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Microwave assisted synthesis of spiro-2,5-diketopiperazines

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Abstract—A general and efficient method for the synthesis of spiro-2,5-diketopiperazines (spiro-DKPs) is described. Cyclization of Boc-protected dipeptides containing spiro-amino acids by microwave assisted heating in water furnished the corresponding spiro-DKPs. The spiro-amino acids were prepared by combining stereoselective alkylation reactions using the Schöllkopf methodology for amino acid construction with Grubbs ring-closing metathesis (RCM) methodology using ruthenium complexes. The RCM reactions and all subsequent transformations to the spiro-DKPs were run with microwave assisted heating, resulting in high yields and short reaction times for all steps.

1. Introduction

Conformationally constrained amino acids (e.g., spiro-amino acids) incorporated into bioactive peptides are useful to both restrict the flexibility of the peptide and to provide information on the topographical requirements of peptide receptors.[1a–e] Moreover, such pseudopeptides display several important advantages compared to natural peptides such as increased bio-stability and improved selectivity toward the natural biological target. Our studies in this field have been focused on the preparation of spiro-2,5-diketopiperazine derivatives (spiro-DKPs). These cyclized dipeptides containing α,α-disubstituted amino acid moieties show a wide range of pharmacological activities,[2a–g] which makes the spiro-DKP substructure an attractive core in medicinal chemistry.

Recently, we reported a general, efficient, and environmentally benign solution phase synthesis of DKPs.[3] The key step in the synthesis was the cyclization of dipeptides using microwave assisted heating in water. We now continue our studies on DKPs to include also sterically congested amino acids. The present study concerns the development of a general method for the preparation of spiro-DKPs from spiro-amino acids. There are few reports in the literature on the synthesis of spiro-DKPs,[4] involving e.g., the direct cyclization of linear substituted dipeptides,[4a–c] the multi-component Ugi-reaction[4e] or other methods.[4d] Our strategy is based on the cyclization of dipeptides containing spiro-amino acids, using microwave assisted heating in water.

2. Results and discussion

The spiro-amino acids were prepared by combining stereoselective alkylation reactions using the Schöllkopf methodology for amino acid construction, which involves the chiral auxiliary (2*R*)-3,6-diethoxy-2,5-dihydro-2-isopropylpyrazine (**1**, Scheme 1), with the Grubbs RCM methodology using ruthenium complexes.[5] The set of synthesized spiro-compounds comprise five-, six- and seven-membered rings containing a double bond (**4a**–**d**, Scheme 1). Bis-alkylation of **1**[6] was performed in a stepwise manner, which enabled the introduction of two different alkene moieties (Scheme 1).[5] The first alkylation reaction was effected by lithiation at −78 °C using *n*-BuLi followed by addition of allyl bromide or 1-bromo-3-butene. Compounds **2a** and **2b** were obtained in 91% isolated yields with a diastereomeric excess (de) of 97% (determined by chiral GC; data not shown). In the second alkylation step the alkylating agent did again approach the reactive carbanionic center at the 5-position from the side of the ring opposite to the isopropyl group. The de values of compounds **3b** and **3c** were >95% (data not shown).

The RCM reactions were performed by addition of catalytic amounts (ca. 1%) of benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium[7] to a solution of bis-olefin **3**. Solvent, reaction time, and temperature were varied in order to find the optimal reaction conditions. The progress of the reactions was monitored by TLC. The cyclization to obtain such spiranes have previously been run in DCE at slightly elevated temperatures for 8–24 h, using the first generation Grubbs catalyst.[5] In the present study Grubbs second generation catalyst was used to improve the efficiency of the reaction, and microwave assisted heating was attempted

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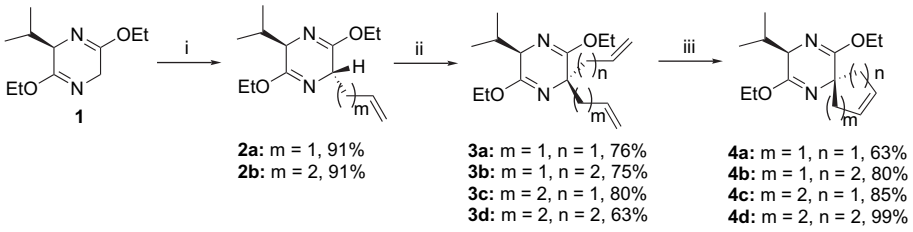


Scheme 1. Preparation of spiro derivatives **4**. Reagents and conditions: (i) *n*-BuLi, THF, R–Br, −78 °C, 16 h; (ii) *n*-BuLi, THF, R′–Br, −78 °C, 16 h; (iii) 1% benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium (Grubbs second generation catalyst), microwave, 140 °C, 15 min, DCM.

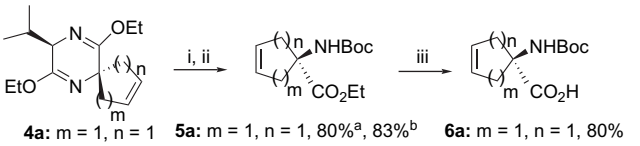
to reduce the reaction time. Spirane **4a** was obtained in 63% isolated yield after 15 min at 140 °C. Shorter reaction times or lower temperatures resulted in incomplete reactions and longer reaction times, e.g., 30 min, gave no further increase in yields. When DCM was used as solvent reaction temperatures of 140 °C and higher could only be achieved after addition of a drop of ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate, BMIM). However, such high temperatures resulted in formation of several side-products. The use of DMF as solvent slowed down the reaction considerably and also resulted in byproduct formation. As a consequence, the syntheses of **4a–d** were carried out using microwave assisted heating at 140 °C for 15 min (Scheme 1). The seven-membered ring structure (**4d**) and the two six-membered rings (**4b** and **4c**) were obtained in high yields (80–99%), whereas formation of the five-membered ring (**4a**) proceeded less readily (63%). The cyclization of **3a** to **4a** is probably more sensitive to steric hindrance than the cyclizations to the larger ring structures. Similar results have been observed by others.[5]

To obtain the free spiro-amino acids the bis-lactim ether **4** has to be hydrolyzed in acid. The hydrolysis of **4a–d** was first carried out using 0.2 M TFA (10 equiv) in MeCN at rt for 5 days as described in the literature.[5] Separation of the two amino acid esters turned out to be difficult, therefore the amino functions of the two amino acids were Boc-protected (Boc-anhydride in THF, reflux) prior to purification. Separation of the two Boc-protected amino acid esters proceeded smoothly and **5a–d** were obtained in 60–78% yields.

In order to reduce the long reaction times the reaction mixture was heated in an oil bath at 60 °C for 20 min using 3.0 equiv of TFA, however, this procedure resulted in incomplete reactions. Extending the reaction time to 1 h resulted in full conversion of the starting material but with concomitant formation of byproducts (data not shown). We then instead tested to run the hydrolysis of **4a–d** using microwave assisted heating. Compound **4a** was dissolved in MeCN (10 mL) and series of experiments were conducted with different amounts of TFA (1.0 and 3.0 equiv), with or without water as co-solvent, at different temperatures (60, 70, 100, and 140 °C) with varying reaction times (10, 15, and 20 min). The reactions were monitored by TLC. Using 1.0 equiv of TFA and water as co-solvent running the reactions at 70, 100 or 140 °C, for 15 or 20 min resulted in complete conversion of the starting material but with formation of several products. However, running the reaction at 60 °C for 20 min using 1.0 equiv of TFA/water resulted in a clean reaction producing only one product with just trace amounts of starting material left. Increasing the amount of TFA to 3.0 equiv gave a complete reaction. Running the same reaction but without water present slowed down the reaction considerably.

Using this optimized protocol for the microwave assisted hydrolysis of **4a–d**, followed by Boc-protection before purification, produced **5a–d** in high yields (80–83%). The differences in yields between the room temperature and the microwave reactions were small for the five-membered ring (**5a**) but significant for the six- and seven-membered rings, 17–22% higher yields were obtained of **5b–d** using microwave heating. Furthermore, the microwave assisted protocol reduced the reaction time from 5 days to 20 min.

Ester hydrolysis of **5a–d** was initially run using aqueous lithium hydroxide (1.0 M) in 1,2-dimethoxyethane at rt for 5 days to give acids **6a–d** (Scheme 2). In order to reduce the reaction time **5b** was heated at 72 °C with 2 equiv of LiOH in 1,2-dimethoxyethane. TLC showed a complete reaction after 12 h but with concomitant formation of byproducts and **6b** was isolated in 75% yield. Our success with applying microwave assisted heating to the ring opening of the bis-lactim ethers, prompted us to also test the ester hydrolysis under microwave conditions. So far, there have been only a few reports describing ester hydrolysis facilitated by microwave assisted heating.[8a,b] Compound **5a** was treated with LiOH in THF/water (1:1 v/v) for 10 min at 60 °C. TLC showed conversion to one product but some starting material remained. Neither an increase of the reaction time to 15 or 30 min nor an increase of the amount of LiOH to 3.0 or 5.0 equiv resulted in complete reactions. Attempts to increase the reaction temperature to 100 °C for 15 min resulted in a mixture of products. Finally, when **5a** was treated with 25 equiv of LiOH in THF/water (1:1 v/v) under microwave assisted heating for 20 min at 60 °C TLC showed full conversion without any byproduct formation. Using this protocol compounds **6a–c** were all obtained in



Scheme 2. Preparation of Boc-protected spiro-amino acids **6**. Reagents and conditions: (i) [a]0.2 M (10 equiv) TFA in MeCN at rt, 5 days; or [b]3 equiv TFA in MeCN/water (1:1 v/v), microwave, 60 °C, 20 min; (ii) Boc_2O, THF, reflux; (iii) 25 equiv LiOH in THF/water (1:1 v/v), microwave, 60 °C, 20 min; [c]50 equiv KOH in THF/water (1:1 v/v), microwave, 60 °C, 30 min.

high yields (Scheme 2). However, hydrolysis of **5d** gave only a 64% yield of **6d**. Increasing the amount of LiOH to 50 equiv did not result in an improved yield. Only when **5d** was treated with 50 equiv of KOH in THF/water (1:1 v/v) under microwave conditions for 30 min at 60 °C a complete reaction was obtained and **6d** could be isolated in 76% yield. Exploration of the general utility of this method in other systems and in multistep sequences is ongoing.

The next step to obtain the spiro-DKPs is the synthesis of dipeptide derivatives containing spiro-amino acids. It is known that the older methods used for peptide bond formation (activation of acids via anhydrides, oxazolones, active esters or carbodiimides) are inefficient with sterically congested amino acid residues like spiro-amino acids.[9] It was therefore no surprise that the Boc-protected dipeptide methyl ester **7d** (Scheme 3) was only obtained in 17% yield when EDC was used as coupling reagent.

In later years there has been an increasing interest in using microwave assisted heating to reduce the reaction time needed to achieve difficult peptide couplings.[10a–h] We decided to investigate if the coupling of our spiro-amino acids could be carried out rapidly and in high yields with microwave conditions using HBTU/HOBt[11] or HATU[12] coupling protocols. Compound **6a** was coupled with PheOMe (1.2 equiv) using HBTU/HOBt (1.2 equiv) or HATU (1.2 equiv) at 60 °C. The reaction had to be run for 30 min to obtain full conversion of the starting materials in the HATU mediated reaction, whereas the HBTU/HOBt coupling still showed traces of starting materials. The HBTU/HOBt coupling resulted in 77% isolated yield of **7a**, and the HATU coupling in 94% yield. For comparison, the same two reactions carried out at rt for 3 h gave 50% isolated yield of **7a** in the HBTU/HOBt coupling and 53% for the HATU coupling. Longer reaction times or higher reaction temperatures did not increase the yields. As a consequence the microwave assisted syntheses of dipeptides **7a–d** were carried out at 60 °C for 30 min. In general, the HBTU/HOBt couplings gave isolated yields in the range 67–77%, while the HATU couplings gave considerably higher yields (89–94%). It should be noted that peptide couplings are often conducted on solid phase using 3- to 4-fold excess of reagents in order to drive the reactions to completion. In this case the reactions were run in solution without any excess of reagents and still very high yields were obtained.

DKPs **8a–d** were synthesized in a microwave reactor via a simultaneous Boc-deprotection and cyclization of **7a–d** using water as solvent. We have previously shown that it is possible to perform such cyclizations of Boc-protected dipeptide methyl esters in excellent yields at 200 °C for 10 min.[3] To investigate if this high temperature was needed also for the formation of spiro-DKPs, a series of test reactions using **7a** for the synthesis of **8a** were performed at different temperatures (140, 160, 180, and 200 °C) and reaction times (5, 10, and 15 min). Reactions at 140 °C for 5 min gave complete Boc-deprotection but resulted in a mixture of **8a** and the linear dipeptide. After 10 min only **8a** was observed. At 160 and 180 °C only traces of the dipeptides were observed whereas running the reaction at 200 °C only required 5 min for complete reaction. To avoid overheating the reaction mixtures the microwave assisted syntheses of the spiro-DKPs **8a–d** were carried out at 160 °C for 10 min. The isolated yields of **8a–d** were in the range 80–86%.

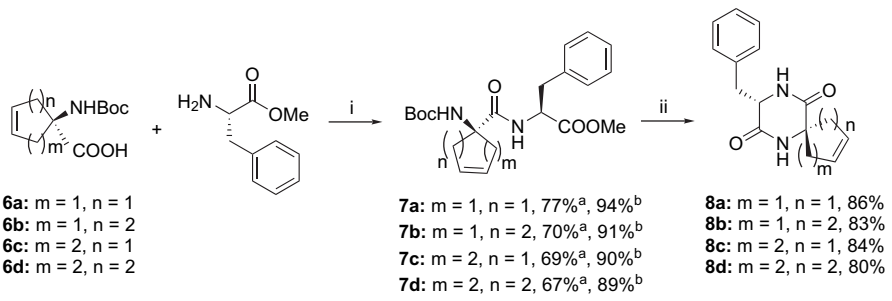
3. Conclusions

In this study we have developed an efficient synthetic procedure for the preparation of spiro-DKPs. The spiro-amino acids were prepared by combining stereoselective alkylation reactions using the Schöllkopf methodology for amino acid construction with the Grubbs RCM methodology using ruthenium complexes. The RCM reactions as well as all subsequent transformations to the spiro-DKPs were run using microwave assisted heating, generating high yields of products in short reaction times. If instead thermal heating was used full conversion of starting materials could be obtained, but several byproducts were formed, which made the purification problematic and reduced the isolated yields of the products. It should be noted that the total reaction times for the ring-closing metathesis, the hydrolysis of the Schöllkopf's chiral auxiliary and the hydrolysis of the amino ethyl esters were only 55 min using microwave assisted heating! The procedure presented here should be useful for the preparation of a large variety of spiro-DKPs, using spiro-amino acids in combination with natural or non-natural amino acids.

4. Experimental

4.1. General

All reagents and solvents were of analysis or synthesis grade. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ if not otherwise



Scheme 3. Preparation of spiro-DKPs **8**. Reagents and conditions: (i) [a]HBTU/HOBt or [b]HATU, DIPEA, DMF, 60 °C, 30 min; (ii) H_2O, microwave, 160 °C, 10 min.

stated on a JEOL JNM-EX 400-spectrometer at 400 and 100 MHz, respectively. The reactions were monitored by thin-layer chromatography (TLC), on silica plated aluminum sheets (Silica gel 60 F_{254}, E. Merck), detecting spots by UV and/or a $KMnO_4$ dip solution followed by heating. Column chromatography was performed on wet packed silica (Silica gel 60 (0.040–0.063 mm), E. Merck) using flash chromatography. Melting points were measured in a Büchi Melting Point B-540 apparatus and were uncorrected. Optical rotations were measured at room temperature with a Perkin–Elmer 341 LC polarimeter. The microwave reactions were carried out using a Biotage Initiator instrument in closed vials. The IR spectra were obtained on a Perkin–Elmer 16 PC spectrometer.

Elemental analyses were performed at Kolbe Mikroanalytisches Laboratorium, Mülheim and der Ruhr, Germany. Compound **1** was prepared according to a literature procedure.[6]

4.2. General procedure for alkylation of the Schöllkopf bis-lactim ethers 1 and 2

n-BuLi (1.2 equiv; 1.6 M in hexane) was added to a solution of **1** or **2** in THF (2 mL/mmol) under N_2 at −78 °C. After 1 h, the alkyl halide was added dropwise over 30 min. The mixture was left to reach rt overnight before the reaction was quenched by addition of phosphate buffer (0.10 M; pH 7; 40 mL). The two phases were separated and the aqueous phase extracted with DCM (2×50 mL). The organic phases were combined, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography on silica gel using EtOAc/hexane (2:98 v/v) as eluant.

4.2.1. (2*R*,5*S*)-5-Allyl-3,6-diethoxy-2,5-dihydro-2-isopropylpyrazine (2a). Compound **1** (4.72 g, 22.3 mmol) and allyl bromide (2.0 mL, 22.9 mmol) were reacted as described in the general procedure to give **2a** as a yellow oil (5.1 g, 91%). R_f 0.20 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 2958, 2363, 1658, 1232, 1035; $[\alpha]_D$ +16.2 (*c* 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.69 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.03 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.25–1.28 (m, 6H, 2×CH_2–CH_3), 2.22–2.29 (m, 1H, C*H*–(CH_3)$_2$), 2.53–2.59 (m, 2H, CH_2–CH=CH_2), 3.86 (d, 1H, *J*= 3.3 Hz, NC*H*), 4.04–4.13 (m, 5H, 2×CH_2–CH_3, NC*H*CH_2), 5.01–5.07 (m, 2H, CH_2–CH=CH_2), 5.63–5.73 (m, 1H, CH_2–C*H*=CH_2); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.5, 17.2, 19.6, 30.7, 44.7, 60.2, 60.4, 60.6, 61.1, 117.4, 133.7, 162.1, 162.8; FAB HRMS [M+H] calcd for $C_{14}H_{25}N_2O_2$ 253.1916, found 253.1913.

4.2.2. (2*R*,5*S*)-5-(3-Butenyl)-3,6-diethoxy-2,5-dihydro-2-isopropylpyrazine (2b). Compound **1** (4.42 g, 20.8 mmol) and 1-bromo-3-butene (2.1 mL, 20.8 mmol) were reacted as described in the general procedure to give **2b** as a yellow oil (5.04 g, 91%). R_f 0.22 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 2960, 2368, 1660, 1236, 1040; $[\alpha]_D$ +8.2 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.69 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.02 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.24–1.26 (m, 6H, 2×CH_2–CH_3), 2.19–2.28 (m, 5H, C*H*–(CH_3)$_2$, CH_2–CH_2–CH=CH_2), 3.87 (d, 1H, *J*=3.3 Hz, NC*H*), 3.99–4.05 (m, 1H, NC*H*CH_2), 4.06–4.16 (m, 4H, CH_2–CH_3), 4.91–5.02 (m, 2H, CH_2–CH_2–CH=CH_2), 5.68–5.85 (m, 1H, CH_2–CH_2–C*H*=CH_2); ^{13}C NMR δ 14.4, 14.5, 17.3, 19.7, 30.7, 39.9, 45.3, 60.2, 60.4, 60.6, 61.1, 117.3, 133.8,162.0, 162.8; FAB HRMS [M+H] calcd for $C_{15}H_{27}N_2O_2$ 267.2072, found 267.2071.

4.2.3. (2*R*)-5,5-Diallyl-3,6-diethoxy-2,5-dihydro-2-isopropylpyrazine (3a). Compound **2a** (2.3 g; 9.2 mmol) and allyl bromide (0.8 mL; 9.2 mmol) were reacted as described in the general procedure to give **3a** as a yellow oil (2.0 g; 76%). R_f 0.30 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 2967, 2945, 2368, 1689, 1234, 1215, 1156, 1037; $[\alpha]_D$ −0.4 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.70 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.05 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.23–1.25 (m, 6H, 2×CH_2–CH_3), 2.21–2.35 (m, 3H, C*H*–(CH_3)$_2$, CH_2–CH=CH_2), 2.83–3.07 (m, CH_2–CH=CH_2), 3.95 (d, 1H, *J*=3.3 Hz, *NC*H), 4.04–4.21 (m, 4H, 2×CH_2–CH_3), 4.9–5.08 (m, 4H, 2×CH_2–CH=CH_2), 5.50–5.64 (m, 2H, 2×CH_2–C*H*=CH_2); ^{13}C NMR ($CDCl_3$) δ 14.3, 14.4, 16.9, 19.4, 31.4, 49.1, 49.4, 60.4, 61.1, 62.5, 117.4, 118.1, 133.6, 134.7, 162.1, 162.8; FAB HRMS [M+H] calcd for $C_{17}H_{29}N_2O_2$ 293.2229, found 293.2225.

4.2.4. (2*R*,5*R*)-5-Allyl-5-(3-butenyl)-3,6-diethoxy-2,5-dihydro-2-isopropylpyrazine (3b). Compound **2a** (2.3 g; 9.2 mmol) and 1-bromo-3-butene (1.1 mL; 11 mmol) were reacted as described in the general procedure to give **3b** as a yellow oil (2.6 g; 75%). R_f 0.41 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 2960, 2942, 2364, 1682, 1230, 1210, 1146, 1024; $[\alpha]_D$ −10.0 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.71 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.04 (d, 3H, *J*= 6.9 Hz, CH–(CH_3)$_2$), 1.23–1.27 (m, 6H, CH_2–CH_3), 1.74–2.71 (m, 7H, CH_2–CH=CH_2, CH_2–CH_2–CH=CH_2, C*H*–(CH_3)$_2$), 3.90 (d, 1H, *J*=3.3 Hz, NC*H*), 4.05–4.12 (m, 4H, 2×CH_2–CH_3), 4.88–4.96 (m, 4H, CH_2–CH_2–CH=CH_2, CH_2–CH=CH_2), 5.62– 5.64 (m, 2H, CH_2–CH_2–C*H*=CH_2, CH_2–C*H*=CH_2); ^{13}C NMR δ 14.3, 14.2, 17.1, 19.4, 21.5, 31.2, 32.8, 36.6, 55.6, 60.2, 60.4, 60.6, 114.15, 117.3, 134.8, 138.6, 160.6, 165.8; FAB HRMS [M+H] calcd for $C_{18}H_{31}N_2O_2$ 307.2385, found 307.2375.

4.2.5. (2*R*,5*S*)-5-Allyl-5-(3-butenyl)-3,6-diethoxy-2,5-dihydro-2-isopropylpyrazine (3c). Compound **2b** (1.2 g; 4.4 mmol) and allyl bromide (0.38 mL; 4.4 mmol) were reacted as described in the general procedure to give **3c** as a yellow oil (0.73 g; 80%). R_f 0.41 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 2960, 2942, 2364, 1682, 1230,1210, 1146, 1024; $[\alpha]_D$ −9.5 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.67 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.07 (d, 3H, *J*= 6.9 Hz, CH–(CH_3)$_2$), 1.20–1.27 (m, 6H, 2×CH_2–CH_3), 1.54–1.94 (m, 7H, CH_2–CH=CH_2, CH_2–CH_2–CH=CH_2, C*H*–(CH_3)$_2$), 3.82 (d, 1H, *J*=3.3 Hz, NC*H*), 4.02–4.32 (m, 4H, 2×CH_2–CH_3), 4.86–4.98 (m, 4H, CH_2–CH_2–CH=CH_2, CH_2–CH=CH_2), 5.70–5.82 (m, 2H, CH_2–CH_2–C*H*=CH_2, CH_2–C*H*=CH_2); ^{13}C NMR ($CDCl_3$) δ 14.4, 14.5, 17.1, 19.6, 28.6, 30.7, 32.8, 39.6, 60.2, 60.4, 60.8, 61.1, 114.0, 114.1, 134.6, 139.1, 162.1, 162.9; FAB HRMS [M+H] calcd for $C_{18}H_{31}N_2O_2$ 307.2385, found 307.2375.

4.2.6. (2*R*)-5,5-Bis(3-butenyl)-3,6-diethoxy-2,5-dihydro-2-isopropylpyrazine (3d). Compound **2b** (1.1 g; 4.1 mmol) and 1-bromo-3-butene (0.42 mL; 4.1 mmol) were reacted as described in the general procedure to give **3d** as a yellow oil (0.83 g; 63%). R_f 0.42 (EtOAc/hexane

2:98); IR ν_{max} (film/cm^{-1}) 2960, 2934, 2356, 1692, 1254, 1232, 1148, 1024; $[\alpha]_D$ −10.2 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.67 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.07 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.20–1.27 (m, 6H, 2×CH$_2$–CH_3), 1.54–1.94 (m, 8H, 2×CH_2–CH_2–CH=CH$_2$), 2.34–2.35 (m, 1H, C*H*–(CH$_3$)$_2$), 3.82 (d, *J*=3.3 Hz, 1H, *NCH*), 4.02–4.32 (m, 4H, 2×CH_2–CH$_3$), 4.86–4.98 (m, 4H, 2×CH$_2$–CH$_2$–CH=CH_2), 5.70–5.82 (m, 2H, 2×CH$_2$–CH$_2$–C*H*=CH$_2$); ^{13}C NMR ($CDCl_3$) δ 14.4, 14.5, 17.1, 19.6, 28.6, 29.2, 30.7, 39.6, 40.2, 60.2, 60.4, 60.8, 61.1, 114.0, 114.1, 134.6, 139.1, 162.1, 162.9; FAB HRMS [M+H] calcd for $C_{18}H_{31}N_2O_2$ 321.2542, found 307.2540.

4.3. General procedure for the synthesis of (2*R*)-2,5-dihydro-2-isopropylpyrazine-5-spiro-cycloalkenes 4a–d

Benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium (Grubbs second generation catalyst) (1%) was added to a solution of **3** in DCM (5 mL). The mixture was heated in a microwave cavity at 140 °C for 15 min (200 W). The solvent was removed under vacuum and the residue was purified by flash chromatography on silica gel using EtOAc/hexane (2:98 v/v) as eluant.

4.3.1. (2*R*)-3,6-Diethoxy-2,5-dihydro-2-isopropylpyrazine-5-spiro(3-cyclopentene) (4a). Compound **3a** (1.6 g; 5.5 mmol) was reacted as described in the general procedure to give **4a** as a yellow oil (0.92 g; 63%). R_f 0.25 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 3628, 3018, 1636, 1458, 1215, 1156; $[\alpha]_D$ −0.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.70 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.05 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.23–1.25 (m, 6H, 2×CH$_2$–CH_3), 2.21–2.35 (m, 3H, C*H*–(CH$_3$)$_2$, CCH_2CH), 2.83–3.07 (m, 2H, CCH_2CH), 3.95 (d, 1H, *J*=3.3 Hz, NC*H*), 4.04–4.21 (m, 4H, 2×CH_2–CH$_3$), 5.50–5.64 (m, 2H, 2×C*H*=); ^{13}C NMR ($CDCl_3$) δ 14.3, 14.4, 16.9, 19.4, 31.4, 49.1, 49.4, 60.4, 61.1, 62.5, 127.8, 128.3, 160.8, 165.3; FAB HRMS [M+H] calcd for $C_{15}H_{25}N_2O_2$ 265.1931, found 265.1933.

4.3.2. (2*R*,5*R*)-3,6-Diethoxy-2,5-dihydro-2-isopropylpyrazine-5-spiro(3-cyclohexene) (4b). Compound **3b** (1.1 g; 3.4 mmol) was reacted as described in the general procedure to give **4b** as a yellow oil (0.76 g; 80%). R_f 0.38 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 3625, 3015, 1634, 1450, 1220, 1156; $[\alpha]_D$+29.5 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.71 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.05 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.22–1.27 (m, 6H, 2×CH$_2$–CH_3), 1.41 (m, 7H, CH_2CH_2CH, CCH_2CHC*H*–(CH$_3$)$_2$), 3.90 (d, 1H, *J*=3.3 Hz, NC*H*), 4.05–4.12 (m, 4H, 2×CH_2–CH$_3$), 5.62–5.64 (m, 2H, 2×C*H*=); ^{13}C NMR ($CDCl_3$) δ 14.2, 14.3, 17.1, 19.4, 21.5, 31.2, 32.8, 36.6, 55.6, 60.2, 60.4, 60.6, 123.8, 126.3, 160.6, 165.8; FAB HRMS [M+H] calcd for $C_{16}H_{27}N_2O_2$ 279.2072, found 279.2078.

4.3.3. (2*R*,5*S*)-3,6-Diethoxy-2,5-dihydro-2-isopropylpyrazine-5-spiro(3-cyclohexene) (4c). Compound **3c** (0.67 g; 2.2 mmol) was reacted as described in the general procedure to give **4c** as a yellow oil (0.52 g; 85%). R_f 0.38 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 3625, 3015, 1634, 1450, 1220, 1156; $[\alpha]_D$ −15.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.69 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.05 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.20–1.26 (m, 6H, 2×CH$_2$–CH_3), 1.73–2.70 (m, 7H, CH_2CH_2CH, CCH_2CHC*H*–(CH$_3$)$_2$), 3.90 (d, 1H, *J*=3.3 Hz, NC*H*), 4.05–4.12 (m, 4H, 2×CH_2–CH$_3$), 5.62–5.64 (m, 2H, 2×C*H*=); ^{13}C NMR ($CDCl_3$) δ 14.2, 14.3, 17.1, 19.4, 21.5, 31.2, 32.8, 36.6, 55.6, 60.2, 60.4, 60.6, 123.8, 126.3, 160.6, 165.8; FAB HRMS [M+H] calcd for $C_{16}H_{27}N_2O_2$ 279.2072, found 279.2078.

4.3.4. (2*R*)-3,6-Diethoxy-2,5-dihydro-2-isopropylpyrazine-5-spiro(4-cycloheptene) (4d). Compound **3d** (0.59 g; 1.9 mmol) was reacted as described in the general procedure to give **4d** as a yellow oil (0.57 g; 99%). R_f 0.42 (EtOAc/hexane 2:98); IR ν_{max} (film/cm^{-1}) 3622, 3034, 1630, 1434, 1232, 1145; $[\alpha]_D$ +0.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.67 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.07 (d, 3H, *J*=6.9 Hz, CH–(CH_3)$_2$), 1.20–1.27 (m, 6H, 2×CH$_2$–CH_3), 1.54–1.94 (m, 8H, 2×CH_2CH_2CH), 2.34–2.35 (m, 1H, C*H*–(CH$_3$)$_2$), 3.82 (d, 1H, *J*=3.3 Hz, NC*H*), 4.02–4.32 (m, 4H, 2×CH_2–CH$_3$), 5.70–5.82 (m, 2H, 2×CH=); ^{13}C NMR ($CDCl_3$) δ 14.4, 14.5, 17.1, 19.5, 23.2, 23.4, 31.1, 38.4, 38.5, 60.2, 60.3, 60.5, 61.1, 131.9, 132.1, 162.1, 162.9; FAB HRMS [M+H] calcd for $C_{17}H_{29}N_2O_2$ 293.2229, found 293.2229.

4.4. General procedure for the preparation of ethyl 1-(*tert*-butoxycarbonylamino)-3-cycloalkene-1-carboxylates (5a–d) (method a)

A solution of **4** in TFA (0.20 M) (100 mL; 25 mmol; 10 equiv) and MeCN (25 mL) was stirred at rt for 5 days, the solution was concentrated to ca. 25 mL and DCM (50 mL) was added. The phases were separated, and the pH of the aqueous phase was brought to ≈10 by addition of saturated aqueous ammonia (ca. 3 mL). The aqueous phase was extracted with DCM (3×50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in THF (25 mL) and di-*tert*-butyldicarbonate (2.0 equiv) was added, the mixture was refluxed for 20 h. The solvent was evaporated and the residue was purified by flash chromatography on silica gel using EtOAc/hexane (1:9 v/v) as eluant.

4.4.1. Ethyl 1-(*tert*-butoxycarbonylamino)-3-cyclopentene-1-carboxylate (5a). Compound **4a** (0.74 g; 2.8 mmol) was hydrolyzed and the crude product (0.69 g) was converted to **5a** using the general procedure described above. Compound **5a** was isolated as a colorless oil (0.56 g; overall yield 80%); R_f 0.10 (EtOAc/hexane 1:9); IR ν_{max} (film/cm^{-1}) 3378, 2978, 1737, 1503; $[\alpha]_D$ +0.1 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, *J*=6.9 Hz, CH$_2$–CH_3), 1.40 (s, 9H, C(CH_3)$_3$), 2.57 (d, 2H, *J*=16 Hz, CCH_2CH), 3.02 (d, 2H, *J*=16 Hz, CCH_2CH), 4.17 (q, 2H, *J*=6.9 Hz, CH_2–CH$_3$), 5.11 (br s, 1H, N*H*), 5.62 (m, 2H, 2×C*H*=); ^{13}C NMR ($CDCl_3$) δ 14.2, 28.3, 44.9, 61.5, 64.0, 74.0, 127.5, 154.9, 174.3. Anal. Calcd for $C_{13}H_{21}NO_4$: C, 61.16; H, 8.29; N, 5.49. Found: C, 61.26; H, 8.47; N, 5.29.

4.4.2. Ethyl (*R*)-1-(*tert*-butoxycarbonylamino)-3-cyclohexene-1-carboxylate (5b). Compound **4b** (1.1 g; 4.2 mmol) was hydrolyzed and the crude product (0.75 g) was converted to **5b** using the general procedure described above. Compound **5b** was isolated as a colorless oil (0.66 g; overall yield 60%). R_f 0.11 (EtOAc/hexane 1:9); IR ν_{max} (film/cm^{-1}) 3373, 2978, 1731, 1504; $[\alpha]_D$ −31 (*c* 1.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, *J*=6.9 Hz, CH$_2$–CH_3), 1.41 (s, 9H,

C(CH_3)$_3$), 1.85–2.61 (m, 6H, CCH_2CH, CCH_2CH_2CH), 4.18 (q, J=6.9 Hz, 2H, CH_2–CH_3), 4.75 (br s, 1H, NH), 5.54–5.71 (m, 2H, 2×CH=); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.8, 28.3, 31.1, 31.9, 33.9, 56.9, 61.1, 72.1, 122.5, 127.1, 154.9, 174.1. Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.28; H, 8.49; N, 5.03.

4.4.3. Ethyl (*S*)-(*tert*-butoxycarbonylamino)-3-cyclohexene-1-carboxylate (5c). Compound **4c** (0.50 g; 1.9 mmol) was hydrolyzed and the crude product (0.44 g) was converted to **5c** using the general procedure described above. Compound **5c** was isolated as a yellow oil (0.20 g; overall yield 60%). R_f 0.11 (EtOAc/hexane 1:9); IR ν_{max} (film/cm^{-1}) 3373, 2978, 1731, 1504; $[\alpha]_D$ +30 (*c* 1.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J=6.9 Hz, CH_2–CH_3), 1.41 (s, 9H, C(CH_3)$_3$), 1.85–2.61 (m, 6H, CCH_2CH, CCH_2CH_2CH), 4.18 (q, 2H, J=6.9 Hz, CH_2–CH_3), 4.75 (br s, 1H, NH), 5.54–5.71 (m, 2H, 2×CH=); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.8, 28.3, 31.1, 36.5, 56.9, 61.1, 72.1, 122.5, 127.1, 154.9, 174.1. Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.33; H, 8.54; N, 5.09.

4.4.4. Ethyl 1-(*tert*-butoxycarbonylamino)-3-cycloheptene-1-carboxylate (5d). Compound **4d** (0.55 g; 1.9 mmol) was hydrolyzed and the crude product (0.45 g) was converted to **5d** using the general procedure described above. Compound **5d** was isolated as a yellow oil (0.49 g; overall yield 67%). R_f 0.13 (EtOAc/hexane 1:9); IR ν_{max} (film/cm^{-1}) 3372, 2977, 1711, 1510; $[\alpha]_D$ −0.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, J=6.9 Hz, CH_2–CH_3), 1.41 (s, 9H, C(CH_3)$_3$), 1.92–2.16 (m, 8H, 2×CCH_2CH_2CH), 4.18 (q, 2H, J=6.9 Hz, CH_2–CH_3), 4.75 (br s, 1H, NH), 5.68–5.71 (m, 2H, 2×CH=); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.8, 23.3, 27.5, 28.3, 34.3, 61.3, 62.4, 80.0, 127.0, 131.0, 154.8, 174.4. Anal. Calcd for $C_{15}H_{25}NO_4$: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.66; H, 8.47; N, 5.06.

4.5. General procedure for the preparation of ethyl 1-(*tert*-butoxycarbonylamino)-3-cycloalkene-1-carboxylates (5a–d) using microwave assisted heating (method b)

To a solution of **4** in MeCN/H_2O (1:1) (10 mL) was added TFA (3.0 equiv) and the mixture was heated in a microwave cavity at 60 °C for 20 min (125 W). The solution was concentrated to ca. 2 mL and DCM (50 mL) was added. The phases were separated, and the pH of the aqueous phase was brought to ≈10 by addition of saturated aqueous ammonia (0.50 mL). The aqueous phase was extracted with DCM (3×50 mL). The organic phase was dried ($MgSO_4$) and concentrated. The residue was dissolved in THF (25 mL), di-*tert*-butyldicarbonate (2.0 equiv) was added and the mixture was refluxed for 20 h. The solvent was removed under vacuum and the residue was purified by flash chromatography on silica gel using EtOAc/hexane (1:9 v/v) as eluant.

4.5.1. Ethyl 1-(*tert*-butoxycarbonylamino)-3-cyclopentene-1-carboxylate (5a). Compound **4a** (0.48 g; 1.8 mmol) was converted to **5a** (0.41 g; 83%). R_f 0.10 (EtOAc/hexane 1:9).

4.5.2. Ethyl (*R*)-1-(*tert*-butoxycarbonylamino)-3-cyclohexene-1-carboxylate (5b). Compound **4b** (0.50 g; 1.8 mmol) was converted to **5b** (0.40 g; 82%). R_f 0.11 (EtOAc/hexane 1:9).

4.5.3. Ethyl (*S*)-(*tert*-butoxycarbonylamino)-3-cyclohexene-1-carboxylate (5c). Compound **4c** (0.65 g; 2.3 mmol) was converted to **5c** (0.50 g; 80%). R_f 0.11 (EtOAc/hexane 1:9).

4.5.4. Ethyl 1-(*tert*-butoxycarbonylamino)-3-cycloheptene-1-carboxylate (5d). Compound **4d** (0.60 g; 2.0 mmol) was converted to **5d** (0.54 g; 81%). R_f 0.13 (EtOAc/hexane 1:9).

4.6. General procedure for hydrolysis of ethyl esters 5

To a solution of **5** in THF/H_2O (1:1 v/v) (5 mL) was added LiOH (25 equiv) and the mixture was heated in a microwave cavity at 60 °C for 20 min (120 W). The solution was concentrated to ca. 2 mL and DCM (20 mL) was added. The phases were separated, and the pH of the aqueous phase was brought to ≈3 by addition of aqueous citric acid (10%) (50 mL). The aqueous phase was extracted with DCM (2×20 mL). The organic phase was dried ($MgSO_4$). The solvent was removed and the residue was purified by flash chromatography using MeOH/DCM (1:9 v/v) as eluant.

4.6.1. 1-(*tert*-Butoxycarbonylamino)-3-cyclopentene-1-carboxylic acid (6a). Compound **5a** (40 mg; 0.19 mmol) gave **6a** as a white solid (35 mg; 80%). R_f 0.3 (MeOH/DCM 1:9); IR ν_{max} (KBr/cm^{-1}) 3439, 1640; mp 115–117 °C; $[\alpha]_D$ +0.1 (*c* 1, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.40 (s, 9H, C(CH_3)$_3$), 2.60 (d, 2H, J=16 Hz, CCH_2CH), 3.02 (d, 2H, J=16 Hz, CCH_2CH), 5.14 (br s, 1H, NH), 5.62 (s, 2H, 2×CH=); ^{13}C NMR (CD_3OD) δ 27.3, 44.5, 57.7, 71.4, 127.3, 156.3, 174.1. Anal. Calcd for $C_{11}H_{17}NO_4$: C, 58.14; H, 7.54; N, 6.16. Found: C, 58.10; H, 7.62; N, 6.08.

4.6.2. (*R*)-1-(*tert*-Butoxycarbonylamino)-3-cyclohexene-1-carboxylic acid (6b). Compound **5b** (50 mg; 0.18 mmol) gave **6b** as a yellow solid (40 mg; 90%). R_f 0.32 (MeOH/DCM 1:9); IR ν_{max} (KBr/cm^{-1}) 3445, 1653; mp 146–148 °C; $[\alpha]_D$ −7 (*c* 1.15, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.41 (s, 9H, CH_3–Boc), 1.82–2.61 (m, 6H, CCH_2CH, CCH_2CH_2CH), 4.84 (br s, 1H, NH), 5.54–5.71 (m, 2H, 2×CH=); ^{13}C NMR (CD_3OD) δ 21.5, 27.4, 29.4, 33.3, 56.9, 78.8, 122.8, 126.0, 156.1, 174.4. Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.81. Found: C, 59.84; H, 7.87; N, 5.70.

4.6.3. (*S*)-(*tert*-Butoxycarbonylamino)-3-cyclohexene-1-carboxylic acid (6c). Compound **5c** (30 mg; 0.11 mmol) gave 24 mg (93%) of **6c** as a yellow solid. R_f 0.32 (MeOH/DCM 1:9); IR ν_{max} (KBr/cm^{-1}) 3445, 1653; mp 145 °C; $[\alpha]_D$ +8 (*c* 1.20, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.41 (s, 9H, C(CH_3)$_3$), 1.82–2.61 (m, 6H, CCH_2CH, CCH_2CH_2CH), 4.83 (br s, 1H, NH), 5.54–5.71 (m, 2H, 2×CH=); ^{13}C NMR (CD_3OD) δ 21.5, 27.4, 29.4, 33.3, 56.9, 78.8, 122.8, 126.0, 156.1, 174.2. Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.81. Found: C, 59.65; H, 7.86; N, 5.65.

4.6.4. 1-(*tert*-Butoxycarbonylamino)-3-cycloheptene-1-carboxylic acid (6d). Compound **5d** (50 mg; 0.18 mmol) gave 30 mg (64%) of **6d** as a yellow oil. When compound **5d** (50 mg; 0.18 mmol) was treated with 50 equiv KOH in THF/H_2O (1:1 v/v) (5 mL) and heated in a microwave cavity

at 60 °C for 30 min, **6d** was obtained in 76% yield. R_f 0.42 (MeOH/DCM 1:9); IR ν_{max} (film/cm^{-1}) 3442, 1649; $[\alpha]_D$ +0.1 (*c* 1, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 1.40 (s, 9H, C(C*H*$_3$)$_3$), 1.92–2.16 (m, 8H, 2×CC*H*$_2$C*H*$_2$CH), 4.73 (br s, 1H, N*H*), 5.68–5.71 (m, 2H, 2×C*H*=); ^{13}C NMR (CD_3OD) δ 21.8, 23.3, 27.5, 34.3, 28.3, 61.3, 80.0, 127.0, 131.0, 154.7, 174.4. Anal. Calcd for $C_{13}H_{21}NO_4$: C, 61.16; H, 8.29; N, 5.49. Found: C, 61.07; H, 8.20; N, 5.38.

4.7. General procedure for amide coupling reactions using HBTU/HOBt (method a) or HATU (method b) to afford 7

The methyl ester of L-Phe HCl, HBTU/HOBt (method a) or HATU (method b) and DIPEA were dissolved in DMF (4 mL). The mixture was heated at 60 °C for 30 min using microwave assisted heating (70 W). The reaction mixture was diluted with DCM (2×20 mL) and extracted with brine (20 mL). The organic layer was concentrated in vacuo and the crude product was purified by flash chromatography on silica gel using MeOH/DCM (5:95 v/v) as eluant.

4.7.1. *N*-[(1-*tert*-Butoxycarbonylamino-cyclopent-3-enyl)carbonyl]-L-phenylalanine methyl ester (7a). Compound **6a** (36 mg; 0.16 mmol), PheOMe HCl (41 mg; 0.19 mmol), HBTU (72 mg; 0.19 mmol), HOBt (26 mg; 0.19 mmol) and DIPEA (0.16 mL; 0.73 mmol) or HATU (73 mg; 0.19 mmol) and DIPEA (94 μL; 0.54 mmol) were reacted as described in the general procedure. The crude product was purified to give pure **7a** as a white solid. Method a (48 mg; 77%) and method b (59 mg; 94%). R_f 0.5 (MeOH/DCM 5:95); IR ν_{max} (KBr/cm^{-1}) 3445, 1682, 1687; mp 174–175 °C; $[\alpha]_D$ +19.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H, C(C*H*$_3$)$_3$), 2.67–2.88 (m, 4H, 2×CC*H*$_2$CH), 3.00–3.14 (m, 2H, C*H*$_2$-Ph), 3.67 (s, 3H, OC*H*$_3$), 4.80–4.86 (m, 1H, HNC*H*C), 5.46 (s, 1H, N*H*), 5.63 (m, 2H, 2×C*H*=), 6.70 (d, 1H, *J*=7.3 Hz, N*H*–amide), 7.15–7.28 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 28.4, 38.0, 43.9, 52.3, 53.3, 64.5, 79.9, 127.2, 128.6, 129.0, 129.3, 136.0, 154.6, 172.0, 174.1. Anal. Calcd for $C_{21}H_{28}N_2O_5$: C, 64.93; H, 7.27; N, 7.21. Found: C, 64.82; H, 7.30; N, 7.41.

4.7.2. (*R*)-*N*-[(1-*tert*-Butoxycarbonylamino-cyclohex-3-enyl)carbonyl]-L-phenylalanine methyl ester (7b). Compound **6b** (30 mg; 0.12 mmol), PheOMe HCl (32 mg; 0.15 mmol), HBTU (57 mg; 0.15 mmol), HOBt (20 mg; 0.15 mmol) and DIPEA (99 μL; 0.57 mmol) or HATU (57 mg; 0.15 mmol) and DIPEA (73 μL; 0.42 mmol) were reacted as described in the general procedure. The crude product was purified to give pure **7b** as a white solid. Method a (34 mg; 70%) and method b (44 mg; 91%). R_f 0.42 (MeOH/DCM 5:95); IR ν_{max} (KBr/cm^{-1}) 3445, 1680, 1685; mp 162–165 °C; $[\alpha]_D$ −40 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, C(C*H*$_3$)$_3$), 1.87–1.98 (m, 2H, C*H*$_2$–spiro), 2.02–2.12 (m, 2H, C*H*$_2$–spiro), 2.34 (app s, 1H, C*H*$_2$–spiro), 2.50–2.57 (m, 1H, C*H*$_2$–spiro), 3.02–3.10 (m, 1H, C*H*$_2$–Ph), 3.13–3.18 (m, 1H, C*H*$_2$–Ph), 3.69 (s, 3H, OC*H*$_3$), 4.71–4.80 (m, 1H, N*H*–Boc), 4.87 (ddd, 1H, *J*=7.5, 6.0, 5.9 Hz, HNC*H*C), 5.54 (m, 1H, C*H*=), 5.72 (m, 1H, C*H*=), 7.05–7.29 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 21.9, 26.4, 28.3, 34.2, 37.8, 52.3, 53.3, 57.6, 79.9, 122.6, 127.0, 127.7, 128.5, 129.4, 136.2, 155.0, 172.0, 174.0. Anal. Calcd for $C_{22}H_{30}N_2O_5$: C, 65.65; H, 7.51; N, 6.96. Found: C, 65.51; H, 7.62; N, 6.93.

4.7.3. (*S*)-*N*-[(1-*tert*-Butoxycarbonylamino-cyclohex-3-enyl)carbonyl]-L-phenylalanine methyl ester (7c). Compound **6c** (30 mg; 0.12 mmol), PheOMe HCl (32 mg; 0.15 mmol), HBTU (57 mg; 0.15 mmol), HOBt (20 mg; 0.15 mmol) and DIPEA (99 μL; 0.57 mmol) or HATU (57 mg; 0.15 mmol) and DIPEA (74 μL; 0.42 mmol) were reacted as described in the general procedure. The crude product was purified to give pure **7c** as a white solid. Method a (33 mg; 69%) and method b (43 mg; 90%). R_f 0.42 (MeOH/DCM 5:95). IR ν_{max} (KBr/cm^{-1}) 3445, 1680, 1685; mp 165–167 °C; $[\alpha]_D$ +5.3 (*c* 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 9H, C(C*H*$_3$)$_3$), 1.87–1.97 (m, 2H, CC*H*$_2$CH$_2$CH), 2.02–2.12 (m, 2H, CC*H*$_2$C*H*$_2$CH), 2.34 (app s, 1H, CC*H*$_2$CH), 2.50–2.57 (m, 1H, CC*H*$_2$CH), 3.02–3.10 (m, 1H, C*H*$_2$–Ph), 3.13–3.18 (m, 1H, C*H*$_2$–Ph), 3.66 (s, 3H, OC*H*$_3$), 4.71–4.81 (m, 1H, N*H*C), 4.87 (ddd, 1H, *J*=7.3, 6.2, 5.8 Hz, HNC*H*C), 5.54 (m, 1H, C*H*=), 5.72 (m, 1H, C*H*=), 7.05–7.29 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 21.9, 26.4, 28.3, 34.2, 37.8, 52.3, 53.3, 57.6, 79.9, 122.6, 127.0, 127.7, 128.5, 129.4, 136.2, 155.0, 172.0, 174.0. Anal. Calcd for $C_{22}H_{30}N_2O_5$: C, 65.65; H, 7.51; N, 6.96. Found: C, 65.46; H, 7.44; N, 67.08.

4.7.4. *N*-[(1-*tert*-Butoxycarbonylamino-cyclohept-3-enyl)carbonyl]-L-phenylalanine methyl ester (7d). Compound **6d** (30 mg; 0.12 mmol), PheOMe HCl (32 mg; 0.15 mmol), HBTU (57 mg; 0.15 mmol) and HOBt (20 mg; 15 mmol) and DIPEA (99 μL; 57 mmol) or HATU (57 mg; 0.15 mmol), and DIPEA (73 μL; 0.42 mmol) were reacted as described in the general procedure. The crude product was purified to give pure **7d** as a white solid. Method a (33 mg; 67%) and method b (44 mg; 89%). R_f 0.31 (MeOH/DCM 5:95); IR ν_{max} (KBr/cm^{-1}) 3443, 1684, 1688; mp 152–154 °C; $[\alpha]_D$ +2.5 (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, C(C*H*$_3$)$_3$), 1.53–2.18 (m, 8H, 2×CC*H*$_2$C*H*$_2$CH), 3.09–3.10 (m, 2H, C*H*$_2$–Ph), 3.69 (s, 3H, OC*H*$_3$), 4.82 (br s, 1H, N*H*C), 4.85–4.89 (m, 1H, HNC*H*C), 5.65 (m, 2H, 2×C*H*=), 7.12–7.26 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 28.3, 23.4, 33.8, 34.3, 38.2, 52.2, 53.3, 62.9, 80.2, 127.0, 128.5, 129.3, 130.8, 131.1, 136.2, 154.5, 172.1, 174.2. Anal. Calcd for $C_{23}H_{32}N_2O_5$: C, 66.32; H, 7.74; N, 6.73. Found: C, 66.26; H, 7.68; N, 6.78.

4.8. General procedure for synthesis of diketopiperazines 8

Dipeptide **7** was dissolved in water (3 mL) and the reaction mixture was heated in a microwave cavity for 10 min at 160 °C (300 W). The solvent was evaporated and the residue was purified by flash chromatography on silica gel using MeOH/DCM (5:95 v/v) as eluant (**8b–d**) or by filtration (**8a**).

4.8.1. (*S*)-8-Benzyl-6,9-diazaspiro[4.5]dec-2-ene-7,10-dione (8a). Compound **7a** (50 mg; 0.13 mmol) was reacted as described in the general procedure. The solvent was evaporated, the mixture was re-suspended in water and the solid was filtered off to give pure **8a** as a white solid (28 mg;

86%). R_f 0.38 (MeOH/DCM 5:95); IR ν_{max} (KBr/cm^{-1}) 3440, 1682; mp 236–238 °C; $[\alpha]_D$ −62 (*c* 1, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.27 (dd, 1H, *J*=17.1, 2.1 Hz, CC*H*$_2$CH), 2.18 (dt, 1H, *J*=17.6, 2.3 Hz, CC*H*$_2$CH), 2.40 (dd, 1H, *J*=17.2, 2.2 Hz, CC*H*$_2$CH), 2.97 (dd, 1H, *J*=14.0, 4.0 Hz, C*H*$_2$–Ph), 3.04 (dt, 1H, *J*=16.8, 2.4 Hz, CC*H*$_2$CH), 3.26 (dd, 1H, *J*=14.0, 4.0 Hz, C*H*$_2$–Ph), 4.34 (t, 1H, *J*=4.2 Hz, HNC*H*C), 5.57–5.50 (m, 2H, 2×CH═), 7.33–7.19 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 27.3, 39.2, 56.5, 63.3, 126.0, 126.9, 127.1, 128.3, 130.5, 135.3, 166.7, 171.9. Anal. Calcd for $C_{15}H_{16}N_2O_2$: C, 70.29; H, 6.26; N, 10.93. Found: C, 70.35; H, 6.27; N, 11.01.

4.8.2. (3*S*,6*R*)-3-Benzyl-1,4-diazaspiro[5.5]undec-8-ene-2,5-dione (8b). Compound **7b** (50 mg; 0.12 mmol) was reacted as described in the general procedure. The crude product was purified to give **8b** as a white solid (28 mg; 83%). R_f 0.41 (MeOH/DCM 5:95); IR ν_{max} (KBr/cm^{-1}) 3444, 1685; mp 246–248 °C; $[\alpha]_D$ −44 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.49 (d, 1H, *J*=15.0 Hz, CC*H*$_2$CH), 1.79 (dd, 1H, *J*=13.2, 5.9 Hz, CC*H*$_2$CH), 1.96–2.07 (m, 1H, CC*H*$_2$CH$_2$CH), 2.14–2.24 (m, 2H, CCH$_2$C*H*$_2$CH), 2.69 (d, 1H, *J*=17.9 Hz, CC*H*$_2$CH$_2$CH), 2.98 (dd, 1H, *J*=13.9, 8.2 Hz, CH$_2$–Ph), 3.38 (dd, 1H, *J*=13.9, 3.7 Hz, C*H*$_2$–Ph), 4.28 (dd, 1H, *J*=8.4, 3.7 Hz, HNC*H*C), 5.54–5.61 (m, 1H, C*H*═), 5.68–5.77 (m, 1H, C*H*═), 5.94 (s, 1H, N*H*), 6.21 (s, 1H, N*H*), 7.21–7.34 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 20.9, 31.4, 39.6, 45.4, 55.9, 56.4, 123.0, 126.0, 127.7, 129.2, 129.8, 135.3, 166.9, 170.8. Anal. Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.10; H, 6.70; N, 10.37.

4.8.3. (3*S*,6*S*)-3-Benzyl-1,4-diazaspiro[5.5]undec-8-ene-2,5-dione (8c). Compound **7c** (40 mg; 99 μmol) was reacted as described in the general procedure. The crude product was purified to give **8c** as a white solid (23 mg; 84%). R_f 0.44 (MeOH/DCM 5:95); IR ν_{max} (KBr/cm^{-1}) 3444, 1685; mp 250–252 °C; $[\alpha]_D$ +2 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.49 (d, 1H, *J*=15.2 Hz, CC*H*$_2$CH), 1.81 (dd, 2H, *J*=13.1, 6.2 Hz, CC*H*$_2$CH), 1.98–2.06 (m, 1H, CC*H*$_2$CH$_2$CH), 2.15–2.22 (m, 2H, CCH$_2$C*H*$_2$CH), 2.71 (d, 1H, *J*=17.9 Hz, CC*H*$_2$CH$_2$CH), 3.01 (dd, 1H, *J*=13.9, 8.2 Hz, C*H*$_2$–Ph), 3.41 (dd, 1H, *J*=13.9, 3.7 Hz, C*H*$_2$–Ph), 4.30 (dd, 1H, *J*=8.4, 3.7 Hz, HNC*H*C), 5.52–5.60 (m, 1H, C*H*═), 5.63–5.71 (m, 1H, C*H*═), 6.03 (s, 1H, N*H*), 6.23 (s, 1H, N*H*), 7.23–7.36 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 20.7, 31.4, 35.4, 40.6, 56.2, 56.8, 123.1, 126.2, 127.5, 129.2, 129.9, 135.4, 165.9, 170.5. Anal. Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.12; H, 6.75; N, 10.31.

4.8.4. (*S*)-3-Benzyl-1,4-diazaspiro[5.6]dodec-9-ene-2,5-dione (8d). Compound **7d** (50 mg; 0.12 mmol) was reacted as described in the general procedure. The crude product was purified to give **8d** as a white solid (27 mg; 80%). R_f 0.37 (5:95 MeOH:DCM); IR ν_{max} (KBr/cm^{-1}) 3447, 1683; mp 266–268 °C; $[\alpha]_D$ +21 (*c* 0.5, AcOH); ^1H NMR ($CDCl_3$) δ 1.35–2.45 (m, 8H, 2×CC*H*$_2$C*H*$_2$CH), 2.95–3.42 (m, 2H, C*H*$_2$-Ph), 4.25–4.30 (m, 1H, HNC*H*C), 4.82 (br s, 1H, N*H*), 5.66–5.67 (m, 2H, 2×CH═), 6.25 (br s, 1H, N*H*), 7.11–7.35 (m, 5H, Ph–*H*); ^{13}C NMR ($CDCl_3$) δ 23.4, 33.6, 34.3, 38.2, 52.2, 53.3, 61.1, 127.6, 128.5, 129.1, 130.8, 135.2, 166.8, 171.1. Anal. Calcd for $C_{17}H_{20}N_2O_2$: C, 71.81; H, 7.09; N, 9.85. Found: C, 71.92; H, 7.20; N, 9.81.

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