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ORIGINAL ARTICLE

A stereoselective synthesis of α-deuterium-labelled (*S*)-α-amino acids

Elaine O'Reilly · Daniele Balducci · Francesca Paradisi

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Abstract An atom-efficient and stereoselective synthesis has been developed for the preparation of α-^{2}H-labelled (*S*)-α-amino acids, starting from a novel chiral diketopiperazine scaffold. Efficient mono-alkylation of the chiral template afforded the (*S*)-substituted adducts with the nature of the electrophile significantly effecting the stereochemical outcome. Subsequent alkylation was totally selective producing the 1,4-*cis* adduct as the sole diastereoisomer. The deprotection was carried out using cerium ammonium nitrate followed by acid hydrolysis affording the enantipure α-amino acids.

Keywords (*S*)-Deuterated-α-amino acids · Diketopiperazine · Asymmetric synthesis

Introduction

Deuterium-labelled amino acids have become valuable biochemical tools due to their contribution to the field of proteomics. Incorporation of these isotopically labelled and stable amino acids into peptides can assist in protein structure elucidation through spectroscopic methods such as mass spectrometry (Ong et al. 2002; Veenstra et al. 2000) and NMR (Nakanishi et al. 2002; Sattler and Fesik 1996; Takeuchi et al. 2007), thus giving insight into their secondary and tertiary structure. Labelled compounds also offer huge potential in the elucidation of enzymatic mechanisms and biochemical processes (Bachmann et al. 2004; Ducho et al. 2009; Holding and Spencer 2008; Moran et al. 2000).

There are limited examples in literature of the stereoselective and atom-efficient synthesis of α-^{2}H-labelled amino acids, which, when commercially available, are extremely expensive. Previous approaches to their preparation include the deuterogenation of an unsaturated precursor containing a chiral auxiliary (Oba et al. 1998), employment of a suitable deuterated reducing agent (Oba et al. 2006; Pearce et al. 2000), rearrangement of amino cyclopropanone hydrates (Felpin et al. 2000) and isotopic exchange (Rose et al. 1995).

Diketopiperazines (DKPs) have been effectively utilised for the synthesis of chiral amino acids because these diverse building blocks can be easily functionalised and hydrolysed to the corresponding amino acids. They offer a simple and often stereoselective means of incorporating the amino acid functionality. Orena et al. (1992) obtained both the (*S*)- and the (*R*)-alanine, optically pure, by alkylation of the chiral synthon (*S*)-3-methylpiperazine-2,5-dione, with total diastereoselectivity, whilst a good diastereoselection was achieved by alkylating the (*R*)-isomer. Porzi and Sandri then extensively studied the stereocontrolled alkylation of chiral DKPs to obtain natural α-amino acids with generally high diastereoselectivity (Orena et al. 1993; Porzi and Sandri 1994; Paradisi et al. 2000a, b, 2002; Ferioli et al. 2002; Piccinelli et al. 2003). Recently, Balducci et al. (2009a), through an interesting application of the preceding methodology, have achieved the synthesis of α-methyl-amino acids with good stereoselectivity and, in particular, a highly efficient stereocontrolled synthesis of (*S*)-2′,6′-dimethyltyrosine (Balducci et al. 2009b), a non natural α-amino acid that is a component of the δ-opioid antagonist

E. O'Reilly · D. Balducci (✉) · F. Paradisi (✉)
UCD School of Chemistry and Chemical Biology,
Centre for Synthesis and Chemical Biology,
Belfield, Dublin 4, Ireland
e-mail: francesca.paradisi@ucd.ie

D. Balducci
e-mail: daniele.balducci@ucd.ie

Dmt-Tic pharmacophore present in many biologically active compounds (δ antagonists, δ agonist and δ antagonists/μ agonists). Bull et al. (1998a) employed a DKP bearing a chiral proline auxiliary for the synthesis of (*R*)-alanine via C(6)-alkylation of the corresponding lithium enolate.

The synthesis of (*S*)-α-amino acids was achieved by the conjugate addition of organocuprates to the related dihydroalanine compound (Bull et al. 1998b). The same group later reported on the use of the same chiral auxiliary for the preparation of quaternary α-amino acids via alkylation a mono-lactam ether template (Davies et al. 2007). Schöllkopf pioneered the use of a bislactim template bearing a chiral isopropyl auxiliary for the asymmetric synthesis of chiral amino acids (Schöllkopf et al. 1979, 1891). He found that a base-induced alkylation on the bislactim template proceeded with high asymmetric induction due to the directing effect of the auxiliary. Although the use of the chiral isopropyl auxiliary gives impressive stereoselection at the C(6) position in all of the above cases, the nature of this auxiliary at C(3) means upon hydrolysis a valine by-product is generated. This by-product can prove very difficult to separate from the desired amino acid and it is often necessary to convert the amino acids mixture to the corresponding esters and separate them by column chromatography or distillation (Davies et al. 2007: Bull et al. 2007; Smith et al. 2006).

Herein, we report a novel, atom-efficient and stereoselective synthesis of α-^{2}H-labelled α-amino acids, which employs the chiral synthon **1** (Scheme 1), previously developed by our group (O'Reilly et al. 2009, 2010). Porzi and Sandri (1994) exploited a similar chiral synthon for the synthesis of α-amino acids by alkylating on the DKP ring and found that it promoted good diastereoselectivity (ds). The same chiral DKP was later exploited for the synthesis of (*S*)-α-methyl amino acids (Balducci et al. 2009a) and gave good stereoselectivity on alkylation. Initially, attempts were made to introduce the deuterium onto the α-carbon of the disubstituted DKP by using two equivalents of lithium bis(trimethylsilyl)amide (LHMDS) and quenching with ^2H_2O. However, as Rose et al. (1995) found, this method resulted in low selectivity and the formation of by-products. The procedure was then adapted, allowing for the incorporation of deuterium through a highly efficient isotopic exchange. The *N*-protecting group employed functions as a chiral auxiliary, which can direct the stereochemistry on alkylation, resulting in a diastereomeric excess (de) of up to 66%. The use of this *N*-protecting group also allows for its cleavage under mild conditions by employing cerium ammonium nitrate (CAN), allowing retention of the DKP scaffold and increasing the spectrum of amino acids achievable using this synthetic strategy.

Results and discussion

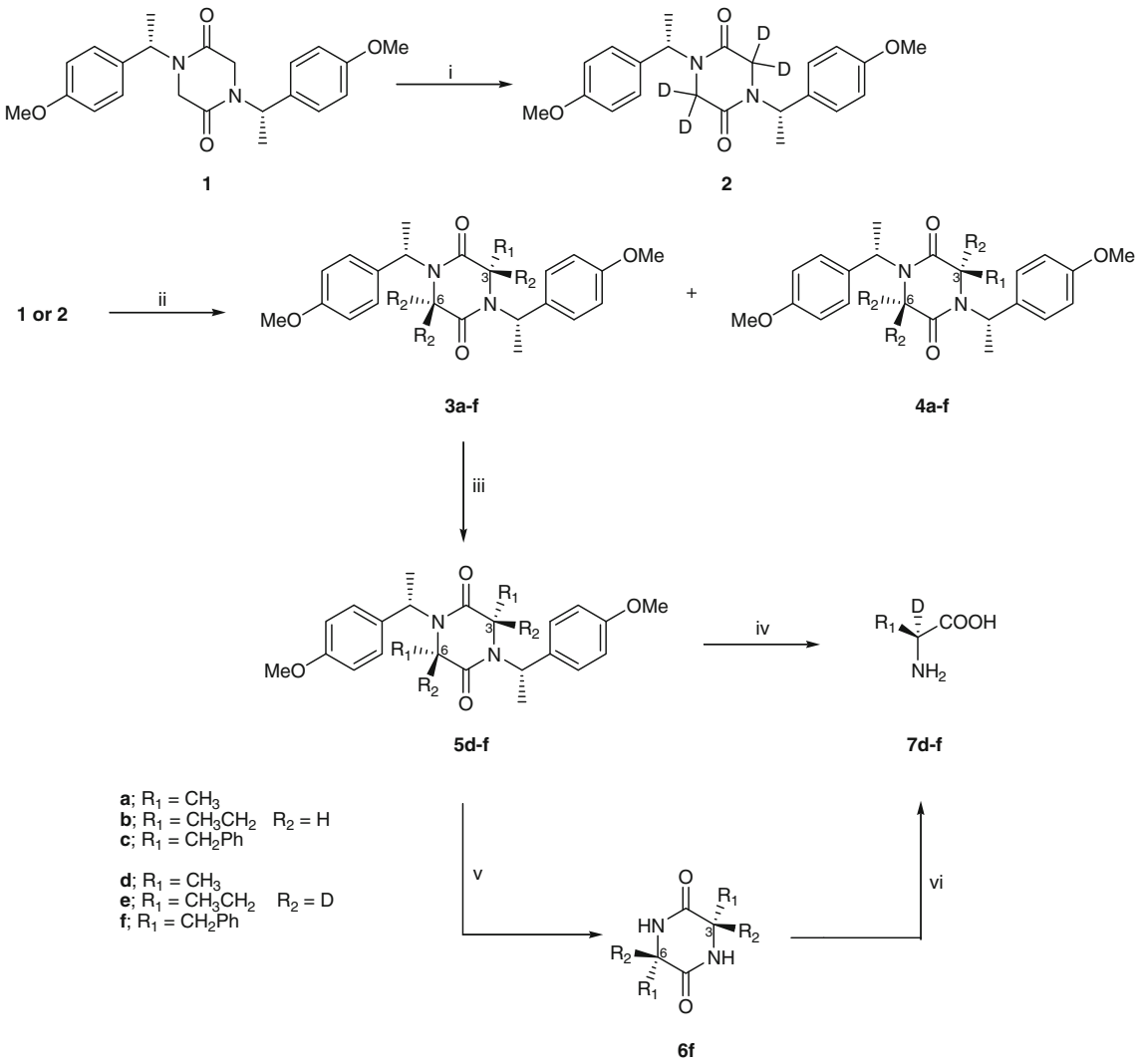
The chiral 1,4-bis[(*S*)-1-phenylethyl]piperazine-2,5-dione **1** has recently been synthesised with excellent yields under phase transfer conditions (O'Reilly et al. 2009, 2010). Previous work on a similar chiral synthon showed that it directed the first alkylation with good ds, with the second alkylation on the (*S*)-mono-substituted adduct being totally selective producing solely the 1,4-*cis* products (Porzi and Sandri 1994).

The aim of this work was to introduce bulk on the *N*-alkyl auxiliary with the aim of enhancing the selectivity in the first alkylation. In this context, the chiral synthon **1** has been employed for the synthesis of a series of α-deuterium-labelled (*S*)-α-methyl-α-amino acids. This chiral template was subjected to a highly efficient isotopic exchange and after treatment with NaOH (1.0 eq) in CH_3OD, the 3,6-deuterated DKP **2** was isolated with high isotopic purity (≥95%). A comparative study was carried out on both the labelled and non-labelled templates.

Mono-alkylation of templates **1** and **2**

Efficient C(3)-alkylation of **1** and **2** was achieved using 1.0 eq of LHMDS, when the enolate was quenched with 1.0 eq of the appropriate electrophile, benzyl bromide, ethyl iodide and methyl iodide, affording the (3*S*)-mono-alkylated products in high yields and with good ds in most cases. The stereochemistry of selected compounds (**3b** and **4d**) was determined by ^{1}H NMR NOESY experiments. A marked ^{1}H NMR shielding pattern was observed for the (3*R*) and (3*S*)-mono-alkylated derivatives and these patterns were exploited to assign the stereochemistry of the other compounds by analogy. The de of all the compounds was determined by ^{1}H NMR analysis of the crude mixture. The stereochemistry of the final amino acids was then confirmed by comparison with previously reported optical rotation values.

There was no notable distinction in the selectivity attained when using DKP templates **1** or **2**. Efficient C(3)-alkylation was achieved with all three electrophiles with the selectivity strongly dependent on the steric contribution from the alkyl halide. No selectivity was observed on quenching the enolate of **1** or **2** with methyl iodide. The 50:50 mixture of **3a:4a** and **3d:4d** isomers obtained was easily separated by column chromatography. Modest selectivity was observed for the C(3)-alkylation of templates **1** and **2** with ethyl iodide, affording the (3*S*) products **3b** and **3e**, respectively, with a de of 20%. Alkylation of both templates with benzyl bromide furnished the (3*S*)-isomers **3c** and **3f** in a de of 66%, an increase of 16% on the previously reported chiral synthon **1**.



Scheme 1 (i) NaOH, CH_3OD, reflux; (ii) and (iii) LHMDS, anhyd. THF, R-X; (iv) HI, reflux then Dowex resin (**5d** and **5e**) (Balducci et al. 2009b and references therein); (v) CAN, CH_3Cl_2/H_2O (3:2) (**5f**). (vi) HCl, reflux then Dowex resin

Alkylation of mono-substituted templates **3a–f**, **4a** and **4d**

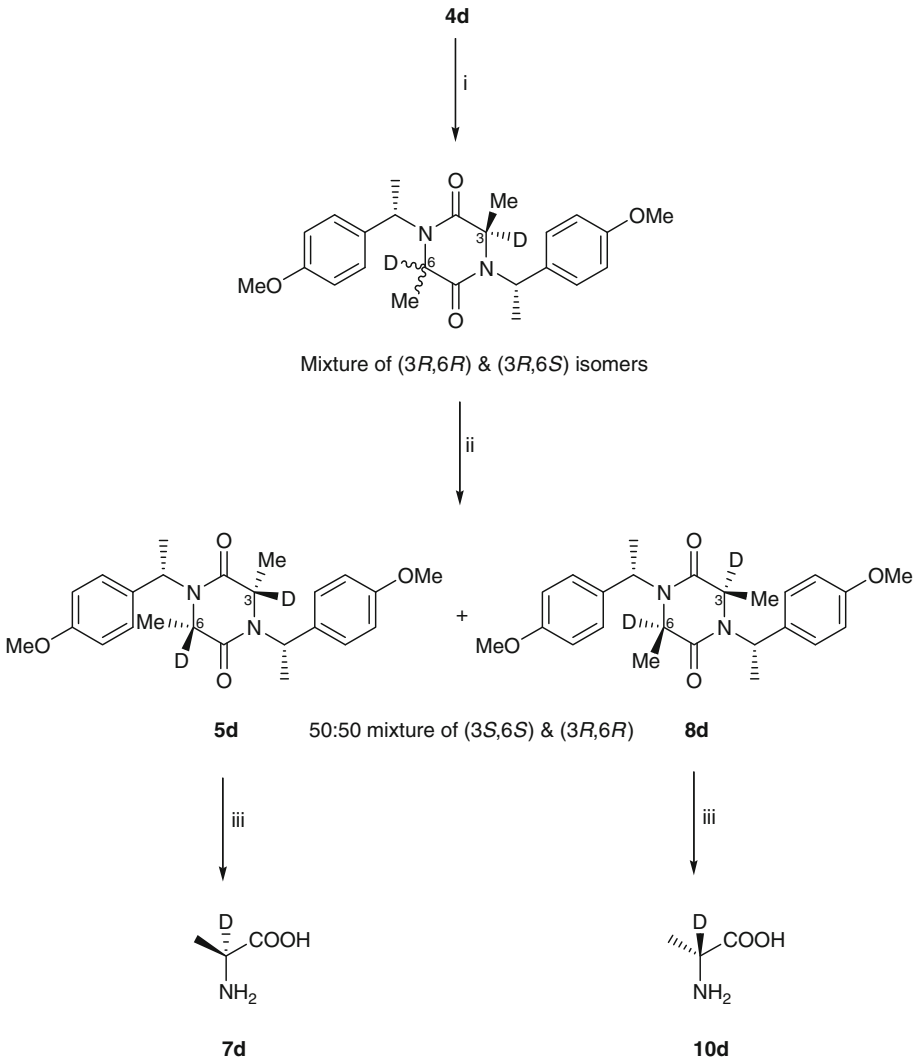
C(6)-alkylation of the (*S*)-mono-substituted adducts **3a–f** was highly selective (>98%) in each case, yielding the (3*S*,6*S*) dialkyl derivatives **5a–f** as the sole diastereoisomer. In the case of the methyl derivatives, a small quantity of tri-alkylated product had formed and was inseparable from the **5b** and **5e** isomers. Consequently, full characterisation of these two compounds and the amino acid (2*S*)-[2-2H]-2-aminobutanoic acid, which resulted from the hydrolysis of **5e**, was not possible.

It is worth noting that C(6)-alkylation of the (3*R*) mono-substituted derivatives **4** gives a mixture of (3*R*,6*R*) and (3*R*,6*S*) isomers, which when treated under basic conditions without any purification, isomerise, yielding a 50:50 mixture of (3*R*,6*R*) and (3*S*,6*S*) products, as demonstrated in Scheme 2. This phenomenon was first observed by Porzi and Sandri (1994). Incorporation of this isomerisation step into the synthesis means the yield of the (3*S*,6*S*) isomers can be dramatically increased and up to a further 25% of the target isomer is attainable.

The diastereoisomers **5d** and **8d** were easily separated by chromatography and the (3*S*,6*S*) isomer hydrolysed to the corresponding (*S*)-amino acid **7d**. This step compensates for the relatively modest stereoselectivity in the C(3)-alkylation. The (3*R*,6*R*) isomer **8d** was also hydrolysed, yielding the (*R*)-amino acid **10d**.

Deprotection and hydrolysis of templates **5d–f**

The nature of the chiral directing group on this novel template allows for mild deprotection using CAN and retention of the substituted DKP scaffold or, alternatively,



Scheme 2 (i) LHMDS, anhyd. THF, CH_3I; (ii) NaOH, CH_3OD; (iii) HI, reflux then Dowex resin (Balducci et al. 2009b and references therein)

for a one-pot deprotection and hydrolysis of the substituted DKPs to the corresponding α-amino acids (Scheme 1).

The di-substituted templates **5d** and **5e** were deprotected and hydrolysed in one-pot in refluxing HI, yielding the corresponding α-^{2}H-labelled (S)-α-amino acids **7d** and **7e** in quantitative yields after purification on Dowex resin (Balducci et al. 2009b and references therein).

Treatment of **5f** with CAN yielded deprotected DKP **6f**, which was subjected to a mild acid hydrolysis affording the enantiopure α-^{2}H-labelled (*S*)-α-amino acid **7f** that was purified on Dowex resin.

In both cases, the symmetrical nature of the template allows for exceptional ease of purification, which is not the case when a non-symmetrical template is used where the directing group is present on the C(6) carbon. In these cases, it is often necessary to convert the amino acid salts to the corresponding methyl esters followed by distillation or chromatography (Davies et al. 2007), where an overall maximum yield of 50% is attainable.

Conclusion

A short and efficient stereoselective synthesis of α-^{2}H-labelled (*S*)-α-amino acids has been developed, which can give a de of up to 66% when a bulky electrophile is employed. The modest selectivity in the first alkylation can be compensated for by the isomerisation of the mixture of (3*R*,6*R*) and (3*R*,6*S*)-substituted templates, which results from the alkylation of the (3*R*)- mono-substituted derivative **4d**, allowing a theoretical 25% of the (3*S*,6*S*) isomer to be recovered. We have demonstrated that the (3*R*,6*R*)-substituted templates can be hydrolysed to the corresponding (*R*)-α-amino acids, which are valuable synthetic and biologically important entities. The *N*-alkyl substituent, which acts as a chiral directing group, also allows for mild deprotection with retention of the DKP scaffold and its symmetric nature leads to a straightforward purification of the target amino acids. This synthetic approach can potentially be employed for the preparation of a wide

range of α-^{2}H-labelled (*S*)-α-amino acids, giving access to important biological tools that are often commercially inaccessible or expensive.

Experimental section

General methods

Proton and carbon nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR, respectively) were recorded on 300 MHz (operating frequencies: ^{1}H, 299.88 MHz; ^{13}C, 75.41), 400 MHz (operating frequencies: ^{1}H, 399.75 MHz; ^{13}C, 101.00), 500 MHz (operating frequencies: ^{1}H, 499.72 MHz; ^{13}C, 125.65) and 600 MHz (operating frequencies: ^{1}H, 599.78 MHz; ^{13}C, 150.82) FT spectrometers. Tetramethylsilane was used as an internal reference in the deuterated chloroform ($CDCl_3$) ($\delta = 0.00$ ppm) for ^{1}H NMR spectra. The middle $CDCl_3$ solvent peak was referenced to 77.02 ppm for ^{13}C NMR spectra. Dioxane was used as an internal reference in the deuterated water (D_2O) ($\delta = 3.75$ ppm) for ^{1}H NMR spectra and ($\delta = 67.19$ ppm) for ^{13}C NMR. The residual dimethyl sulfoxide peak was referenced when applicable ($\delta = 2.54$ ppm) for the ^{1}H NMR spectra and ($\delta = 40.45$ ppm) for ^{13}C NMR. The coupling constants (*J*) are in Hz and the chemical shifts (δ) are given in parts per million. High-resolution mass spectra were obtained on a Waters/Micromass instrument. Optical rotation values were measured at 20°C on a Perkin-Elmer 241 polarimeter. Melting points are uncorrected. Evaporation in vacuo refers to the removal of solvent on a Büchi rotary evaporator with an integrated vacuum pump. Thin-layer chromatography (TLC) was carried out on aluminium-backed 60 F254 silica gel.

For synthesis and spectroscopic data and characterisation of compound **1**, see O'Reilly et al. 2009, 2010.

1,4-Bis[(S)-[3-^{2}H,6-^{2}H]-1-phenylethyl]piperazine-2,5-dione ***2***

DKP **1** (1.0 eq) and NaOH (1.0 eq) was stirred in refluxing CH_3OD for 4 h. The solvent was removed in vacuo and water was added. The solution was extracted with ethyl acetate (×3) and the organic fractions combined, dried over $MgSO_4$ and concentrated. The product was isolated as a white solid of 90% yield with no further purification. M.p: 109.5–110.3°C; (*Rf* = 0.5, EtOAc/hexane, 60:40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, $J = 8.7$ Hz, 4H), 6.86 (d, $J = 8.7$ Hz, 4H), 5.90 (q, $J = 7.1$ Hz, 2H), 3.80 (s, 6H), 1.51 (d, $J = 7.1$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7, 159.3, 130.2, 128.6, 114.1, 55.3, 49.6, 44.2 (t, $J = 20.3$ Hz), 15.3; $[\alpha]_D^{20} = -342.2$ ($c = 1.1$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{22}H_{23}D_4N_2O_4$: 387.2222, found: 387.2222 $[M+H]^+$.

General procedure for the synthesis of compounds ***3****,* ***4*** *and* ***5***

LHMDS (solution in THF, 1.0 eq) was added dropwise to a stirred solution of template **1** or **2** (1.0 eq) in anhydrous THF at −20°C. After 1 h, the mixture was cooled to −78°C and a solution of the alkyl halide (1.0 eq) in anhydrous THF was added dropwise. The reaction mixture was allowed to warm slowly to room temperature and stirred for a further 1 h before being quenched by the addition of water (^2H_2O in the case of the deuterated derivatives). Ethyl acetate was added, the organic layer was separated and the aqueous layer was extracted with ethyl acetate (×3). The organic layers were combined, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by column chromatography.

(S)-1,4-Bis((S)-1-(4-methoxyphenyl)ethyl)-3-methylpiperazine-2,5-dione ***3a***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as an off-white waxy solid of 46% yield after alkylating **1** with iodomethane. (*Rf* = 0.21, EtOAc-hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14–7.11 (m, 4H), 6.89–6.85 (m, 4H), 5.81–5.73 (m, 2H), 3.87–3.75 (m, 8H), 3.60 (d, $J = 16.9$ Hz, 1H), 1.58 (d, $J = 7.2$ Hz, 3H), 1.49–1.46 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 165.0, 159.2, 131.1, 130.7, 128.2, 128.2, 114.2, 114.1, 55.3, 55.3, 52.8, 51.0, 49.4, 44.2, 19.6, 17.5, 15.4; $[\alpha]_D^{20} = -297.5$ ($c = 1.5$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{23}H_{29}N_2O_4$: 397.2127, found: 397.2145 $[M+H]^+$.

(S)-3-Ethyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***3b***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a thick yellow oil of 55% yield after alkylating **1** with iodoethane. (*Rf* = 0.35, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15–7.11 (m, 4H), 6.90–6.85 (m, 4H), 5.84 (q, $J = 7.1$ Hz, 1H), 5.72 (q, $J = 7.2$ Hz, 1H), 3.89 (d, $J = 17.2$ Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.67–3.63 (m, 1H), 3.56 (d, $J = 17.2$ Hz, 1H), 1.98–1.84 (m, 1H), 1.87–1.76 (m, 1H), 1.59 (d, $J = 7.2$ Hz, 3H), 1.47 (d, $J = 7.1$ Hz, 3H), 1.01–0.96 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6, 165.2, 159.3, 159.3, 130.8, 130.7, 128.4, 128.4, 114.2, 114.2, 58.2, 55.3, 55.3, 51.7, 49.5, 44.3, 27.5, 17.5, 15.6, 9.4; $[\alpha]_D^{20} = -330.4$

($c = 0.5$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{24}H_{31}N_2O_4$: 411.2284, found: 411.2284 $[M+H]^+$.

(S)-3-Benzyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***3c***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a white solid of 77% yield after alkylating **1** with benzyl bromide. Mp: 116.3–118°C; ($Rf = 0.51$, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.27 (m, 3H), 7.21 (dd, $J = 7.5$, 1.7 Hz, 2H), 7.14 (d, $J = 8.6$ Hz, 2H), 6.99 (d, $J = 8.6$ Hz, 2H), 6.91–6.86 (m, 2H), 6.83–6.78 (m, 2H), 5.85 (q, $J = 7.3$ Hz, 1H), 5.73 (q, $J = 7.1$ Hz, 1H), 4.05 (m, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 3.30 (dd, $J = 13.8$, 4.2 Hz, 1H), 3.11 (dd, $J = 13.8$, 4.2 Hz, 1H), 3.01 (d, $J = 17.0$ Hz, 3H), 2.14 (d, $J = 17.0$ Hz, 3H), 1.79 (d, $J = 7.3$ Hz, 1H), 1.17 (d, $J = 7.1$ Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 166.1, 159.4, 159.2, 135.3, 131.2, 130.9, 130.4, 128.8, 128.5, 128.1, 127.7, 114.3, 114.0, 58.1, 55.3, 55.3, 52.1, 49.3, 43.6, 40.1, 18.2, 14.7; $[\alpha]_D^{20} = -232.8$ ($c = 0.6$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{29}H_{33}N_2O_4$: 473.2440, found 473.2421 $[M+H]^+$.

(S)-[3-^{2}H,6-$^2H_2$]-1,4-Bis((S)-1-(4-methoxyphenyl)ethyl)-3-methylpiperazine-2,5-dione ***3d***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a clear oil of 45% yield after alkylating **2** with methyl iodide. ($Rf = 0.34$, EtOAc–hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, $J = 8.6$ Hz, 2H), 7.14 (d, $J = 8.6$ Hz, 2H), 6.91–6.82 (m, 4H), 5.82–5.71 (m, 2H), 3.80 (s, 3H), 3.79 (s, 3H),1.54 (d, $J = 2.9$ Hz, 3H), 1.51 (d, $J = 2.9$ Hz, 3H), 0.71 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 164.7, 159.0, 130.9, 130.5, 128.0, 127.9, 113.9, 113.9, 55.1, 55.0, 52.2 (t, $J = 23.2$ Hz), 50.7, 49.2, 44.0–43.4 (m), 19.3, 17.3, 15.2; $[\alpha]_D^{20} = -314.2$ ($c = 1.6$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{23}H_{25}D_3N_2O_4Na$: 422.2135, found: 422.2147 $[M+Na]^+$.

(S)-[3-^{2}H,6-$^2H_2$]-3-Ethyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***3e***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as an oil in 56% yield after alkylating **2** with ethyl iodide. ($Rf = 0.33$, EtOAc–hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–7.10 (m, 4H), 6.93–6.84 (m, 4H), 5.84 (q, $J = 7.1$ Hz, 1H), 5.72 (q, $J = 7.2$ Hz, 1H), 3.81 (s, 6H), 2.02–1.74 (m, 2H), 1.58 (d, $J = 7.2$ Hz, 3H), 1.47 (d, $J = 7.1$ Hz, 3H), 1.03–0.95 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 165.0, 159.2, 159.1, 130.7, 130.6, 114.1, 114.0, 57.7 (t, $J = 21.8$ Hz), 55.2, 55.2, 51.5, 49.3, 44.3–43.6 (m), 27.3, 17.4, 15.5, 9.3; $[\alpha]_D^{20} = 260.1$ ($c = 0.3$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{24}H_{27}D_3N_2O_4Na$: 436.2292, found: 436.2294 $[M+Na]^+$.

(S)-[3-^{2}H,6-$^2H_2$]-3-Benzyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***3f***

After column chromatography (hexane–EtOAc, 5/1), the product was obtained as a white waxy solid of 78% yield after alkylating **2** with benzyl bromide. Mp: 195.2–196.0°C; ($Rf = 0.41$, EtOAc–hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.18 (m, 5H), 7.17–7.12 (m, 2H), 7.02–6.96 (m, 2H), 6.92–6.85 (m, 2H), 6.83–6.77 (m, 2H), 5.85 (q, $J = 7.3$ Hz, 1H), 5.73 (q, $J = 7.0$ Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.29 (d, $J = 13.9$ Hz, 1H), 3.10 (d, $J = 13.9$ Hz, 1H), 1.79 (d, $J = 7.0$ Hz, 3H), 1.17 (d, $J = 7.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 166.00, 159.3, 159.1, 135.1, 131.0, 130.8, 130.3, 128.7, 128.4, 128.1, 127.7, 114.2, 113.9, 57.7 (t, $J = 21.8$ Hz), 55.2, 55.2, 51.9, 49.2, 42.9–43.4 (m), 39.9, 18.2, 14.6; $[\alpha]_D^{20} = -227.2$ ($c = 0.7$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{29}H_{30}D_3N_2O_4$: 476.2629, found: 476.2652 $[M+H]^+$.

(R)-1,4-Bis((S)-1-(4-methoxyphenyl)ethyl)-3-methylpiperazine-2,5-dione ***4a***

After column chromatography (hexane–EtOAc: 3/1), the product was obtained as a white solid of 44% yield after alkylating **1** with iodomethane. Mp: 134–135.3°C; ($Rf = 0.41$, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31–7.27 (m, 2H), 7.18–7.14 (m, 2H), 6.87–6.83 (m, 4H), 5.89–5.83 (m, 2H), 3.99 (q, $J = 7.0$ Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.70–3.61 (d, $J = 17.2$ Hz, 1H), 3.32 (d, $J = 17.2$ Hz, 1H), 1.54–1.51 (m, 6H), 0.72 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 164.7, 159.4, 159.3, 131.3, 130.6, 129.2, 129.1, 128.4, 114.2, 114.0, 55.3, 52.9, 50.3, 49.7, 45.0, 18.0, 16.3, 15.4; $[\alpha]_D^{20} = -360.5$ ($c = 1.3$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{23}H_{29}N_2O_4$: 397.2127, found: 397.2110 $[M+H]^+$.

(R)-3-Ethyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***4b***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a white solid of 37% yield after alkylating **1** with iodoethane. Mp: 107–109.5; ($Rf = 0.45$, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (d, $J = 8.7$ Hz, 2H), 7.17 (d, $J = 8.7$ Hz, 2H), 6.85–6.86 (d, $J = 8.7$ Hz, 2H), 6.85 (d, $J = 8.7$ Hz, 2H) 5.88 (q, $J = 7.1$ Hz, 1H), 5.78 (q, $J = 7.1$ Hz, 1H), 3.90–3.86

(m, 1H), 3.80 (s, 3H), 3.79 (s,3H), 3.64 (d, $J = 17.4$ Hz, 1H), 3.38 (d, $J = 17.4$ Hz, 1H), 1.55 (d, $J = 7.1$ Hz, 3H), 1.51 (d, $J = 7.1$ Hz, 3H), 1.13–1.06 (m, 2H), 0.63–0.59 (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.6, 164.9, 159.4, 159.3, 131.6, 130.7, 129.1, 128.5, 114.1, 113.9, 58.4, 55.3, 51.0, 49.8, 45.0, 25.9, 16.5, 15.4, 9.3; $[\alpha]_D^{20} = -351.5$ ($c = 1.0$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{24}H_{31}N_2O_4$: 411.2284, found: 411.2294 $[M+H]^+$.

(R)-3-Benzyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***4c***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a white solid of 12% yield after alkylating **1** with benzyl bromide. Mp: 190.8–192.2°C; (*Rf* = 0.42, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49–7.46 (m, 2H), 7.09–7.06 (m, 2H), 7.05–7.01 (m, 1H), 6.94 (dd, $J = 9.3$, 2.5 Hz, 2H), 6.92–6.88 (m, 2H), 6.85–6.81 (m, 2H), 6.70 (d, $J = 7.4$ Hz, 2H), 5.90 (q, $J = 7.1$ Hz, 1H), 5.74 (q, $J = 7.1$ Hz, 1H), 4.28 (dd, $J = 5.9$, 3.5 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.31 (d, $J = 17.1$ Hz, 1H), 2.68 (dd, $J = 13.8$, 3.5 Hz, 1H), 2.08–2.02 (m, 2H), 1.58 (d, $J = 7.1$ Hz, 3H), 1.38 (d, $J = 7.1$ Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 165.6, 159.7, 159.3, 134.9, 131.1, 130.2, 129.9, 129.8, 129.2, 128.3, 127.0, 114.2, 113.8, 58.2, 55.4, 55.3, 51.7, 50.4, 44.7, 38.1, 17.1, 16.2; $[\alpha]_D^{20} = -287.0$ ($c = 0.5$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{29}H_{31}N_2O_4$: 471.2284, found: 471.2284 $[M-H]^-$.

(R)-[3-^{2}H,6-$^2H_2$]-1,4-Bis((S)-1-(4-methoxyphenyl)ethyl)-3-methylpiperazine-2,5-dione ***4d***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a white solid of 45% yield after alkylating **2** with methyl iodide. Mp. 133.7–135.4°C; (*Rf* = 0.34, EtOAc–hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, $J = 8.6$ Hz, 2H), 7.14 (d, $J = 8.6$ Hz, 2H), 6.90–6.82 (m, 4H), 5.91–5.80 (m, 2H), 3.80 (s, 3H), 3.79 (s, 3H),1.54 (d, $J = 2.9$ Hz, 3H), 1.51 (d, $J = 2.9$ Hz, 3H), 0.71 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.1, 164.7, 159.4, 159.3, 131.3, 130.7, 129.2, 128.4, 114.2, 114.0, 55.3, 52.5 (t, $J = 22.7$ Hz), 50.3, 49.7, 45.0–44.5 (m), 17.9, 16.4, 15.5; $[\alpha]_D^{20} = -297.6$ ($c = 0.7$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{23}H_{26}D_3N_2O_4$: 400.2316, found: 400.2313 $[M+H]^+$.

(R)-[3-^{2}H,6-$^2H_2$]-3-Ethyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***4e***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a white solid of 36% yield after alkylating **2** with ethyl iodide. Mp: 121.9–123.4; (*Rf* = 0.33, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32–7.29 (m, 2H), 7.19–7.16 (m, 2H), 6.87–6.83 (m, 4H), 5.88 (q, $J = 7.1$ Hz, 1H), 5.78 (q, $J = 7.1$ Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 1.55 (d, $J = 7.1$ Hz, 3H), 1.50 (d, $J = 7.1$ Hz, 3H), 1.13–1.07 (m, 2H), 0.63–0.57 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5, 164.8, 159.2, 159.1, 131.4, 130.6, 129.0, 128.4, 114.0, 113.8, 57.9 (t, $J = 20.7$ Hz), 55.1, 50.8, 49.6, 45.0–44.3 (m), 26.8, 25.7, 16.3, 15.2, 9.2; $[\alpha]_D^{20} = -352.2$ ($c = 0.6$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{24}H_{28}D_3N_2O_3$: 414.2472, found: 414.2459.

(R)-[3-^{2}H,6-$^2H_2$]-3-Benzyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***4f***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a white solid of 13% yield after alkylating **2** with benzyl bromide. Mp: 195.2–196.0°C; (*Rf* = 0.41, EtOAc–hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, $J = 8.5$ Hz, 2H), 7.13–6.79 (m, 9H), 6.69 (d, $J = 7.5$ Hz, 2H), 5.90 (q, $J = 7.2$ Hz, 1H), 5.74 (q, $J = 7.2$ Hz, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 2.68 (d, $J = 13.5$ Hz, 1H), 2.03 (d, $J = 13.5$ Hz, 1H), 1.58 (d, $J = 7.2$ Hz, 3H), 1.38 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.3, 165.6, 159.8, 159.3, 134.9, 131.1, 130.2, 129.9, 129.8, 129.2, 128.3, 127.0, 114.3, 113.8, 57.8 (t, $J = 22.3$ Hz), 55.4, 55.3, 51.7, 50.3, 44.7–44.0 (m), 38.1, 17.1, 16.2; $[\alpha]_D^{20} = -291.3$ ($c = 0.8$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{29}H_{30}D_3N_2O_4$: 476.2629, found: 476.2642 $[M+H]^+$.

(3S,6S)-1,4-Bis((S)-1-(4-methoxyphenyl)ethyl)-3,6-dimethylpiperazine-2,5-dione ***5a***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as an oil of 91% yield after alkylating **3a** with iodomethane. (*Rf* = 0.31, EtOAc–hexane, 60/40); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16–7.09 (m, 4H), 6.90–6.85 (m, 4H), 5.75 (q, $J = 7.2$ Hz, 2H), 3.82 (s, 6H), 3.77 (q, $J = 7.1$ Hz, 2H), 1.61–1.55 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 159.3, 130.8, 128.5, 114.2, 55.3, 52.7, 51.4, 22.1, 17.7; $[\alpha]_D^{20} = -260.1$ ($c = 2.1$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{24}H_{31}N_2O_4$: 411.2284, found: 411.2285 $[M+H]^+$.

(3S,6S)-3,6-Diethyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***5b***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a yellow oil after alkylating **3b** with iodoethane. (*Rf* = 0.60, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12–7.09 (m, 4H), 6.87–6.85 (m, 4H), 5.76 (q, $J = 7.2$ Hz, 2H), 3.81 (s, 6H), 3.53 (dd,

$J = 9.7$, 4.6 Hz, 2H), 1.95–1.84 (m, 4H), 1.57–1.56 (m, 6H), 1.09–1.05 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.0, 159.1, 128.8, 128.3, 114.1, 58.6, 55.2, 51.2, 29.8, 17.7, 11.4; HRMS (ESI): *m/z* calculated for for $C_{26}H_{35}N_2O_4$ 439.2597, found: 439.2597 $[M+H]^+$; The specific rotation and yield of this compound was not measured, due to the compound being insufficiently pure.

(3S,6S)-3,6-Dibenzyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***5c***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a white waxy solid in 90% yield after alkylating **3c** with benzyl bromide. (*Rf* = 0.61, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32–7.22 (m, 6H), 7.12–7.08 (m, 4H), 7.07–7.03 (m, 4H), 6.84–6.81 (m, 4H), 5.70 (q, $J = 7.2$ Hz, 2H), 3.96 (dd, $J = 7.0$, 4.4 Hz, 2H), 3.81 (s, 6H), 2.82 (dd, $J = 14.5$, 4.4 Hz, 2H), 2.70 (dd, $J = 14.5$, 7.0 Hz, 2H), 1.58–1.55 (d, $J = 7.2$ Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 159.3, 137.1, 130.7, 129.6, 128.9, 128.6, 127.0, 114.2, 58.9, 55.3, 52.7, 41.1, 18.2; $[\alpha]_D^{20} = -83.1$ ($c = 0.8$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{36}H_{39}N_2O_4$: 563.2910, found: 563.2919 $[M+H]^+$.

(3S,6S)-[3-^{2}H,6-^{2}H]-1,4-Bis((S)-1-(4-methoxyphenyl)ethyl)-3,6-dimethylpiperazine-2,5-dione ***5d***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as a clear oil of 90% yield after alkylating **3d** with methyl iodide. (*Rf* = 0.30, EtOAc–hexane, 60/40); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15–7.11 (m, 4H), 6.90–6.86 (m, 4H), 5.75 (q, $J = 7.2$ Hz, 2H), 3.82 (s, 6H), 1.61–1.55 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 159.2, 130.8, 128.4, 114.2, 55.3, 52.4 (t, $J = 21.0$ Hz), 51.3, 21.9, 17.7; $[\alpha]_D^{20} = -260.5$ ($c = 1.7$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{24}H_{29}D_2N_2O_4$: 413.2409, found: 413.2389 $[M+H]^+$.

(3S,6S)-[3-^{2}H,6-^{2}H]-3,6-Diethyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***5e***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as an oil after alkylating **3e** with iodoethane. (*Rf* = 0.45, EtOAc–hexane, 60/40); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13–7.08 (m, 4H), 6.88–6.84 (m, 4H), 5.76 (q, $J = 7.2$ Hz, 2H), 3.81 (s, 6H), 1.95–1.82 (m, 4H), 1.56 (d, $J = 7.2$ Hz, 6H), 1.09–1.04 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 159.0, 131.1, 128.2, 113.9, 58.1 (t, $J = 21.2$ Hz), 55.0, 51.0, 30.1, 29.5, 17.5, 11.2; HRMS (ESI): *m/z* calculated for $C_{26}H_{33}D_2N_2O_4$: 441.2722, found: 441.2709 $[M+H]^+$. The specific rotation and yield of this compound was not measured, due to the compound being insufficiently pure.

(3S,6S)-[3-^{2}H,6-^{2}H]-3,6-Dibenzyl-1,4-bis((S)-1-(4-methoxyphenyl)ethyl)piperazine-2,5-dione ***5f***

After column chromatography (hexane–EtOAc, 3/1), the product was obtained as an oil of 91% yield after alkylating **3f** with benzyl bromide. (*Rf* = 0.58, EtOAc–hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.20 (m, 6H), 7.15–7.01 (m, 8H), 6.86–6.79 (m, 4H), 5.75–5.64 (m, 2H), 3.79 (s, 6H), 2.85 (d, $J = 14.5$ Hz, 2H), 2.68 (d, $J = 14.5$ Hz, 2H), 1.56 (d, $J = 7.3$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 159.2, 137.0, 130.6, 129.5, 128.8, 128.5, 127.0, 114.1, 58.5 (t, $J = 20.7$ Hz), 55.2, 52.5, 40.9, 18.1; $[\alpha]_D^{20} = -99.0$ ($c = 0.3$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{36}H_{37}D_2N_2O_4$: 565.3035, found: 565.3027$[M+H]^+$.

(3S,6S)-[3-^{2}H,6-^{2}H]-3,6-Dibenzylpiperazine-2,5-dione ***6f***

DKP **5f** (1.00 g, 1.77 mmol) was dissolved in CH_3CN/H_2O (3:2) and CAN (932 mg, 7.1 mmol) was added. The solution was stirred at room temperature for 2 h. The precipitate was filtered, washed with H_2O and dried in vacuo. Off-white solid; Mp: 305°C (decomp); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.92 (s, 2H), 7.40–7.02 (m, 10H), 2.61 (d, $J = 13.4$ Hz, 2H), 2.27 (d, $J = 12.9$ Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.1, 136.5, 129.8, 128.2, 126.5, 55.3 (t, $J = 15.5$ Hz), 40.1. The specific rotation of this compound was not recorded due to solubility problems.

General procedure for the deprotection and hydrolysis of ***7(d–f)***

Compounds **5d, e** (1.2 mmol) were refluxed in 57% HI (3 mL) for 2 h (Balducci et al. 2009b and references therein). The crude solution was loaded directly onto a column containing the acid ion-exchange resin Dowex 50 WX 8. The resin was washed with distilled water (×5), before eluting the amino acid with NH_4OH (5 M). The aqueous solution was concentrated in vacuo and the pure amino acids were isolated in quantitative yields.

Hydrolysis of ***6f***

Compound **6f** (1.2 mmol) was stirred in refluxing 6 M HCl (3 mL) for 2 h. The crude solution was loaded directly onto a column containing the acid ion-exchange resin Dowex 50 WX 8. The resin was washed with distilled water (×5) before eluting the amino acid with NH_4OH (5 M). The aqueous solution was concentrated in vacuo and the pure amino acid **7f** was isolated in quantitative yield.

(2S)-[2-^{2}H]-Alanine ***7d***

White solid; Mp: 285–287°C; ^{1}H NMR (500 MHz, D_2O) δ 1.53 (s, 3H); ^{13}C NMR (126 MHz, D_2O) δ 175.7, 50.3 (t, $J = 22.7$ Hz), 16.1. $[\alpha]_D^{20} = +13.6$ ($c = 0.8$, 1 M HCl).

(2S)-[2-2H]-2-Aminobutanoic acid ***7e***

White solid; ^{1}H NMR (400 MHz, D_2O) δ 1.94 – 1.83 (m, 2H), 1.02–0.93 (m, 3H); ^{13}C NMR (101 MHz, D_2O) δ 175.5, 56.2 (t, $J = 22.4$ Hz), 24.2, 9.1. The MP, yield and specific rotation values of this compound were not measured, due to the compound being insufficiently pure.

(2S)-[2-^{2}H]-Phenylalanine ***7f***

White solid; Mp: 270°C (decomp) [lit. ref. Mp: 264°C] (Rose et al. 1995); ^{1}H NMR (400 MHz, D_2O) δ 7.47–7.29 (m, 5H), 3.28 (d, $J = 14.5$ Hz, 1H), 3.11 (d, $J = 14.5$ Hz, 1H); ^{13}C NMR (101 MHz, D_2O) δ 174.5, 135.7, 129.98, 129.7, 128.3, 56.3 (t, $J = 22.5$ Hz), 36.9; $[\alpha]_D^{20} = -32.5$ ($c = 0.8$, H_2O) [lit. ref. $[\alpha]_D^{20} = -32.5$] (Gout et al. 1978).

Isomerisation of mixture of (3R,6R) and (3R,6S) isomers yielding ***5d*** *and* ***8d***

The mixture of isomers (413 mg, 1.0 mmol) was dissolved in CH_3OD (3 mL) and NaOH (40 mg, 1.0 mmol) was added. The reaction was stirred under reflux for 2 h. D_2O (5 mL) was added and the solution was extracted with EtOAc (5 × 5 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated in vacuo. The isomers were purified by column chromatography (Hexane–EtOAc, 3/1) yielding (3*S*,6*S*)-[3-^{2}H,6-^{2}H]-1,4-Bis((*S*)-1-(4-methoxyphenyl) ethyl)-3,6-dimethylpiperazine-2,5-dione **5d** (182 mg, 44% yield) as a clear oil and (3*R*,6*R*)-[3-2H,6-2H]-1,4-Bis((*S*)-1-(4-methoxyphenyl)ethyl)-3,6-dimethylpiperazine-2,5-dione **8d** (190 mg, 46% yield) as a white solid. Spectroscopic data for compound **5d** was identical to that reported previously. **8d** M.p: 141.9-143.7°C; (*Rf* = 0.60, EtOAc–hexane, 60/40); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 4H), 6.95–6.81 (m, 4H), 5.80 (q, $J = 7.1$ Hz, 2H), 3.80 (s, 6H), 1.55 (d, $J = 7.1$ Hz, 6H), 0.88 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 159.3, 131.7, 129.1, 113.9, 55.2, 52.8 (t, $J = 21.5$), 50.7, 20.4, 16.2; $[\alpha]_D^{20} = -307.9$ ($c = 1.1$, $CHCl_3$); HRMS (ESI): *m/z* calculated for $C_{24}H_{29}D_2N_2O_4$: 413.2409, found: 413.2419 $[M+H]^+$.

(2R)-[2-^{2}H]-Alanine ***10d***

White solid; Mp: 284–285°C; ^{1}H NMR (500 MHz, D_2O) δ 1.53 (s, 3H); ^{13}C NMR (126 MHz, D_2O) δ 175.7, 50.3 (t, $J = 22.7$ Hz), 16.1; $[\alpha]_D^{20} = -13.8$ ($c = 0.3$, 1 M HCl).

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