JOC The Journal of Organic Chemistry

Note

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J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.6b02895 • Publication Date (Web): 23 Feb 2017

ACS Publications

The Journal of Organic Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

A Comparative Study of the Synthesis, Structural and Physicochemical Properties of Diketopiperazines vs Aza-Diketopiperazines

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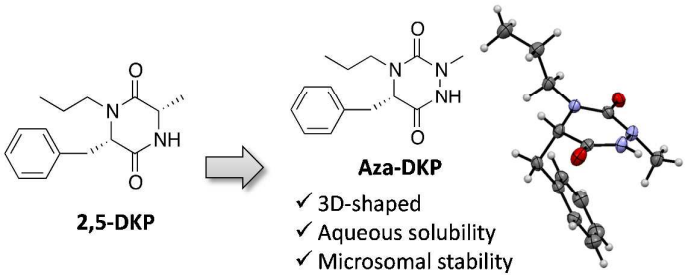


ABSTRACT

Aza-diketopiperazines (Aza-DKP) represent an underprivileged motif obtained by scaffold hopping of 2,5-diketopiperazines (2,5-DKP). Herein, we compare the synthesis but also the structural and the physicochemical properties of aza-DKP **4** *vs* 2,5-DKP **7**. Thus, X-ray and ^{1}H NMR studies show that aza-DKP **4** is a rigid and non-flat scaffold like the 2,5-DKP **7**. Moreover, the replacement of one C_{α}-stereogenic center by a nitrogen atom results in a significant improvement of both the water solubility and the microsomal stability.

High-throughput screening is nowadays one of the most efficient methods to find new leads in medicinal chemistry. Since the advent of combinatorial chemistry associated with solid support synthesis, thousands of derivatives with the same scaffold have been synthesized leading to scarce originality in chemical libraries.[1] Many libraries used in screening are often populated by aromatic compounds which are planar, with low stereochemical complexity.[2] This class of compounds may be suitable to target proteins such as kinases[3] but scaffolds with high content of sp^2 and sp hybridized carbons do not fit optimally with the spatial configuration required for interaction with most biological receptors.[4,5] Moreover, flat aromatic structures are correlated with poorer specificity and physicochemical properties. Lovering *et al.* have shown that compounds possessing a high fraction of sp^3 hybridized carbons (Fsp^3) and chirality have a higher likelihood of progressing through the hit-to-drug process.[6] Therefore, there is a crucial need to enrich the chemical diversity of screening libraries to facilitate the discovery of new drugs. In this context, scaffold hopping approaches consisting in replacing the central core of a known active compound by a new scaffold able to mimic the same biological interactions with the target could be used to improve both pharmacodynamic and pharmacokinetic properties.[7] In the course of the discovery of new scaffolds relevant for medicinal chemistry, we can also list as selection criteria a low molecular weight to allow substitutions while keeping the molecular weight below 500 $g.mol^{-1}$, and the rigidity of these platforms that can adopt a well-defined and three-dimensional (3D) orientation of their substituents. 2,5-Diketopiperazines (2,5-DKP) fulfill these criteria since they have a low molecular weight and a constrained structure providing a non-planar system that directs its four potential substituents in different vectors in 3D-space. 2,5-DKP is a well-known and privileged scaffold in medicinal chemistry as examples are able to interact with many biological targets.[8] While this scaffold has received great attention and is thus described in numerous patents making its exploitation difficult, scaffold hopping by introduction of an

additional nitrogen gives the much less studied heterocycle 1,2,4-triazine-3,6-dione or aza-diketopiperazine (aza-DKP, Figure 1). Indeed, very few efficient syntheses with broad scope are available for the preparation of this rare heterocycle.[9–14] Aza-DKPs have been obtained from N-(mesyloxy)malonamide reacting with hydrazines,[9] by using triphosgene and gaseous HCl to form aza-DKPs from N-Boc hydrazides,[10] from semicarbazone in acidic conditions[11] and by a recent one-pot sequence involving nitrogen-substituted isocyanates as key intermediates.[12] However, these approaches enable the synthesis of disubstituted aza-DKPs only and/or very limited trisubstituted analogs. Thereby, to prepare more complex aza-DKPs and facilitate the preparation of diversity-oriented libraries, our group has recently reported a convenient solution- and solid-phase strategy from α-aminoesters and N-Boc hydrazides,[13] and a step-economical multicomponent process driven by Rh(I)-catalyzed hydroformylation of alkenylsemicarbazides.[14] Interestingly, the replacement of one carbon by a planar nitrogen atom may have a deep influence on the chemical and pharmacochemical properties of DKP. Herein, we evaluate the synthesis and the structural and physicochemical properties of aza-DKP as compared to 2,5-DKP.

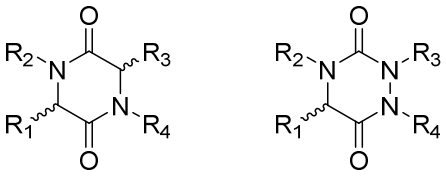
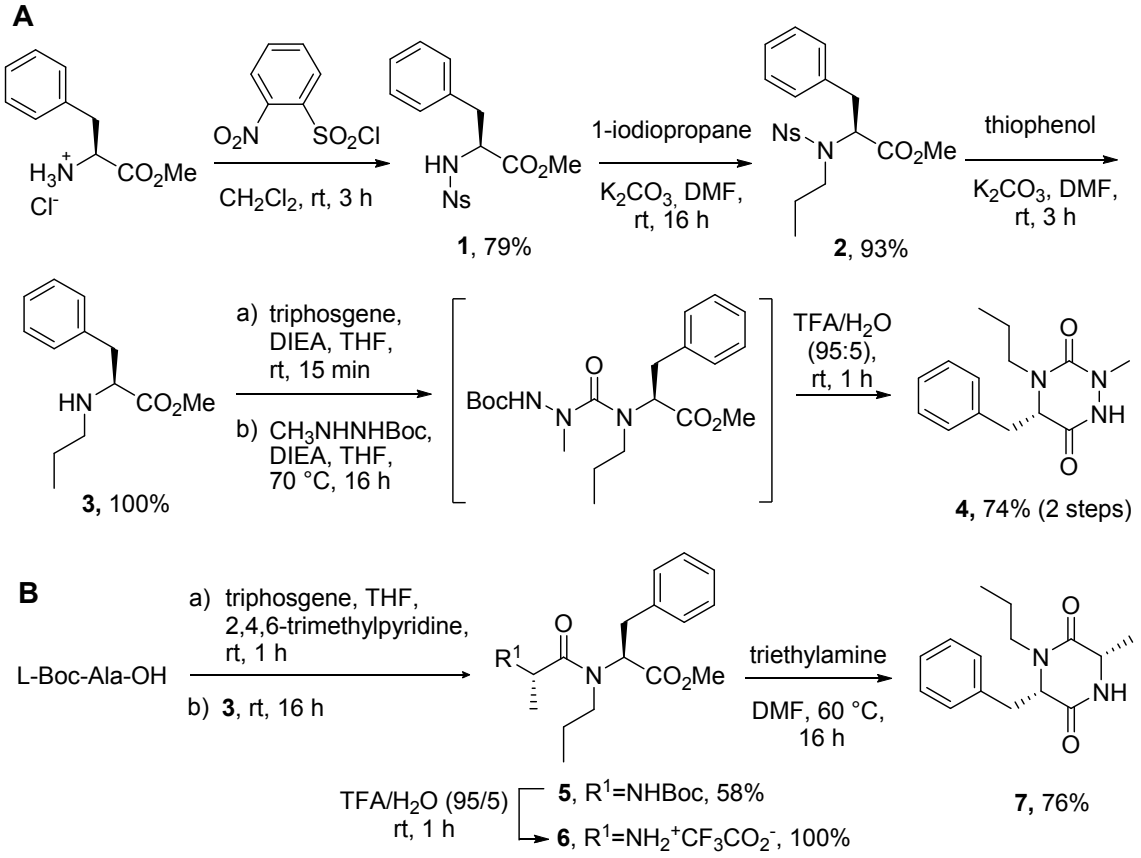


Figure 1. Generic structure of 2,5-DKP and aza-DKP

In order to study the impact of the substitution of the α-carbon by a nitrogen atom on both the physicochemical and structural parameters, we compared aza-DKP **4** and its 2,5-DKP analog **7.** The aza-DKP synthesis began with L-phenylalanine which was first protected using nosyl chloride (Scheme 1). Then, the amine **1** was monoalkylated with propyl iodide under mild conditions using Fukuyama's sulfonamide method.[15] Deprotection of **2** with thiophenol gave

3 with an overall yield of 73% from L-phenylalanine.[16] A subsequent, activation of **3** with triphosgene followed by a treatment with 1-*N*-Boc-2-methylhydrazine provided the corresponding semicarbazide. In the presence of TFA/water (95/5, v/v), the crude mixture was then readily converted into aza-DKP **4** following a deprotection/cyclization process.[14] To synthesize the 2,5-DKP analog, alkyl amine **3** was coupled with *N*-Boc-L-alanine. The resulting linear dipeptide **5** was then treated with a solution of TFA/H_2O (95:5) to initiate the deprotection/cyclization towards the corresponding 2,5-DKP. However, the acidic treatment only resulted in the quantitative deprotection to amine **6** which did not cyclize. This difference of reactivity between the linear dipeptide **5** and the deprotected semicarbazide may be due to the α-effect and lower basicity of the hydrazine moiety leading to a more nucleophilic β-nitrogen that favors the cyclization in acidic catalysis. Nevertheless, a basic treatment with triethylamine in DMF at 60 °C resulted in the cyclization of **6** affording the 2,5-DKP **7** in a good yield (76%).[17] The overall yield for the synthesis of aza-DKP **7** is higher than that obtained for 2,5-DKP **7** (54% *vs* 32% from L-phenylalanine). As compared to 2,5-DKP **7**, the synthesis of aza-DKP **4** is more rapid and performed at room temperature that makes easier the access to chemical libraries.

Scheme 1. Synthesis of aza-DKP **4** (A) and 2,5-DKP **7** (B)



Both aza-DKP **4** and 2,5-DKP **7** were crystalline materials that were suitable for obtaining single-crystal X-ray diffraction structures.[18] Despite the substitution of a carbon by a nitrogen atom in the central core, both compounds clearly display similarities in the spatial orientation of the three substituents in space (Figure 2). However, some subtle structural differences were observed. Indeed, the conformation of the aza-DKP **4** is a flattened pseudo-boat shape with N2-N1-C1-N3 and N2-C2-C3-N3 dihedral angles of -4.2(3)° and -22.2(2)°, respectively whereas the conformation of the 2,5-DKP **7** is a flattened boat shape with N1-C2-C1-N2 and N1-C3-C4-N2 dihedral angles of -10.1(3)° and -11.1(3)°, respectively. In both compounds, the benzyl moiety is in an axial orientation on the same side as the methyl substituent while the propyl group is orientated on the other side to avoid a steric clash.

Moreover, the X-ray structure of aza-DKP **4** shows that the methyl moiety is notably deviated from the N2-N1-C1 plane with an angle of 27.1(2)° and adopts a pseudo-axial position. The propyl moiety linked to N3 is diverted with an angle of 18.7(2)° from the C1-N3-C3 plane and the hydrogen atom on N2 is diverted with an angle of 6.0(2)° from the N1-N2-C2 plane. For the 2,5-DKP **7**, the deviation for the methyl moiety is more important (48.1(2)° from the C1-C2-N1 plane), the other deviations are moderate (2.7(2)° for the propyl moiety from the C1-N2-C4 plane, and 12.2(19)° for the hydrogen atom carried by N1 from the C2-N1-C3 plane) relative to the aza-DKP **4**. Interestingly N1 of the aza-DKP ring adopts a partial sp^3 hybridization as observed for aza-peptides.[19] Examination of the distance between N1 and the plane formed by N2, C1, C2 and C3 indicates a S-configuration with a 0.229(2) Å distortion from planarity.

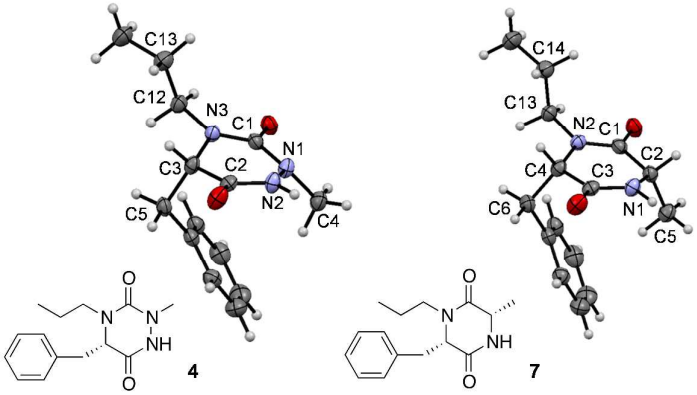


Figure 2. X-ray structures of aza-DKP **4** and 2,5-DKP **7**

The spatial configuration of 2,5-DKP **7** has previously been studied and shown to be a rigid structure.[20] Malavašič *et al.* have published that according to ^{1}H NMR, the chemical shift of the methyl group protons at C2 in 2,5-DKP **7** is unusually low, around 0.8 ppm in DMSO at 298 K, whereas the signal of these protons in 2,5-DKP without the benzyl group appears between 1.40 ppm and 1.54 ppm. These data are in accordance with the crystallographic structure of 2,5-DKP **7** which has displayed a spatial proximity of the methyl protons with the aromatic ring allowing π-σ interaction.[20] In order to investigate the spatial organization of the

aza-DKP **4**, a ^{1}H NMR study was performed in $CDCl_3$, a non-polar solvent, and in DMSO, a polar aprotic solvent (Figure 3).

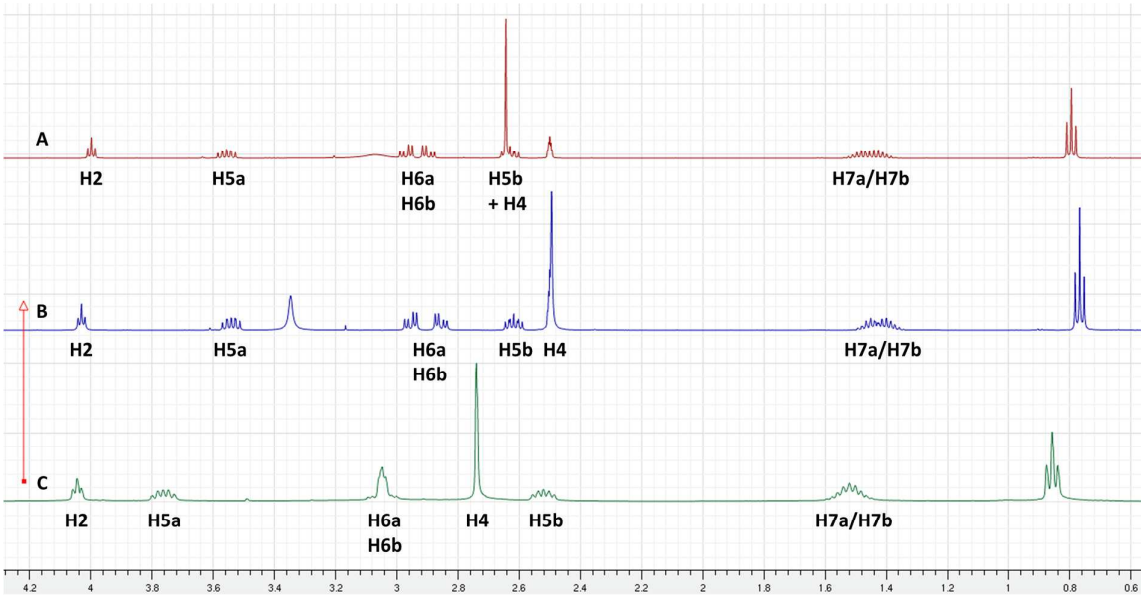


Figure 3. ^{1}H NMR spectra of aza-DKP **4** in DMSO at 363 K (A), in DMSO at 298 K (B) and in $CDCl_3$ at 298 K (C). .

In the spectra, the two protons H12 (H12a and H12b) of the propyl chain appear as an AX_2Y spin system. Furthermore, their chemical shifts were significantly different as their signals were at 3.75 ppm and 2.51 ppm in $CDCl_3$ and at 3.55 ppm and 2.62 ppm in DMSO at 298 K. The increase of the temperature from 298 K to 363 K had no effect on the chemical shift of these protons. Thus, these two protons are in a different magnetic environment indicating that the rotation of the propyl group is slow on the NMR time-scale. In addition, the chemical shift of the methyl group (H4) is shifted to low field (2.73 ppm in $CDCl_3$ and 2.49 ppm in DMSO at 298 K). As observed for 2,5-DKP **7,** this could be explained by shielding of these protons by the proximal benzyl moiety. The chemical shift of these methyl protons was slightly affected by the increase of temperature (2.49 ppm at 298 K to 2.64 ppm at 363 K).

Thus, the nature of the solvent and the temperature have a low impact on the chemical shift of the protons and consequently on the conformation of the aza-DKP scaffold. According to our

X-ray and NMR data, aza-DKP **4** displays a rigid structure that prevents the propyl chain rotation and blocks the methyl group on the same side as the benzyl ring. Thus, the ability of aza-DKP scaffold to efficiently orientate its substituents in different planes of 3D-space should be an advantage for drug discovery by optimizing interaction with biological targets.[21] On the other hand, in comparison to DKP, the aza-DKP scaffold may undergo adaptive chirality as previously reported.[22]

Regarding the drug-like properties, aza-DKP **4** and 2,5-DKP **7** have a low polar surface area and molecular weight, less than five hydrogen bond acceptors and only one hydrogen bond donor in accordance with Lipinski rules and other guidelines for oral bioavailability.[23] Some physicochemical properties of aza-DKP **4** and 2,5-DKP **7** were then assessed (Table 1). Thermodynamic solubility studies in a pH 7.4 PBS buffer showed that the additional nitrogen atom on aza-DKP **4** had a significant effect on the compound solvation. Indeed, a 17-fold increase was observed on the aqueous solubility of aza-DKP **4** as compared to 2,5-DKP **7** (16.5 mM vs 0.98 mM, respectively). Moreover, $logD_{7.4}$ was lowered enabling the introduction of non-polar substitutions on this scaffold while keeping an acceptable distribution coefficient during the optimization process for drug discovery purposes.

Table 1. Drug-like Properties and Physicochemical Parameters of aza-DKP **4** and 2,5-DKP **7**

Parameters	Aza-DKP **4**	2,5-DKP **7**
Molecular weight (g/mol)	261.3	260.3
Polar surface area (Å²)[a]	52.65	49.41
H-bond acceptor	5	4
H-bond donor	1	1
Rotatable bond	4	4
Solubility (mM)[b]	16.5 ± 0.6	0.98 ± 0.06
$LogD_{7.4}$	1.06 ± 0.03	1.38 ± 0.13

[a]Polar surface area calculated by ChemAxon software. [b]Thermodynamic solubility measured in a pH 7.4 phosphate-buffered saline.

The chemical and metabolic stabilities of Aza-DKP **4** and 2,5-DKP **7** were also studied (Table 2). After 24 h of incubation in PBS buffer, these two compounds were fully stable. Similarly, Aza-DKP **4** and 2,5-DKP **7** remained perfectly intact in plasma after 6 h of exposure in spite of the presence of amidases. In mouse liver microsomes, the aza-DKP **4** was found to be more stable than the 2,5-DKP **7** after 30 min of incubation (83% vs 51% remaining) indicating the biological robustness of this new scaffold.

Table 2. Chemical and Metabolic Stability of aza-DKP **4** and 2,5-DKP **7**

Stability[a]	Aza-DKP **4** **%**	2,5-DKP **7** **%**
PBS[b]	> 98	> 98
Plasma[c]	> 98	> 98
Microsomes[d]	83 ± 3	51 ± 1

[a]Percent of remaining starting compounds. [b]pH 7.4 phosphate-buffered saline after 24 h. [c]Mouse plasma after 6 h at 37 °C. [d]Mouse hepatocytes after 30 min at 37 °C.

In conclusion, the comparison between 2,5-DKP and aza-counterpart offers an interesting insight regarding the synthesis, the conformation and pharmacokinetic properties of the latter. Relying on an efficient and rapid deprotection/cyclization process, aza-DKP was obtained at room temperature and with a higher yield than 2,5-DKP. As shown by X-ray analysis and ^{1}H NMR study, aza-DKP displayed a structure close to 2,5-DKP despite the replacement of one tetrahedral carbon by a nitrogen atom. Interestingly, this aza-heterocycle exhibited a rigid backbone directing its substituents in different directions of 3D-space. This non-planar scaffold provides a new opportunities to improve drug-target interactions and success in clinical studies.[6] Furthermore, the presence of the nitrogen atom significantly improved aqueous solubility and distribution coefficient compared to its 2,5-DKP analog allowing the substitution with more hydrophobic groups during the optimization process to increase the biological activity. Metabolic stability was also significantly enhanced thanks to this scaffold

as compared to the 2,5-DKP analog. In summary, we demonstrated that aza-DKP is an original heterocycle, readily accessible from simple building blocks, giving new opportunities in medicinal chemistry by mimicking the structural architecture of 2,5-DKP while it may improve patentability and physicochemical and pharmacokinetic properties.

EXPERIMENTAL SECTION

General methods. Reagents, dry solvents and deuterated solvents were obtained from commercial sources and used without any further purification. Thin-layer chromatography was performed on silica gel 60 F_{254} plates.

Flash chromatography was performed on silica gel pre-packed columns RP18 (30 mm). Analytical reverse-phase high-performance liquid chromatography (RP-HPLC) separations were performed on a C18 column (2.7 μm, 4.6 mm x 75 mm) using a linear gradient (5 % to 95 % of solvent B in solvent A in 7 min, flow rate 1.6 mL/min), detection at 220 nm, solvent A : water/0.1 % trifluoroacetic acid (TFA), solvent B : acetonitrile/0.1 % TFA).

Semi-preparative reverse-phase high-performance liquid chromatography (RP-HPLC) separations were performed on a RP-C18 column (7 μm, 19 mm x 300 mm).

^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz/125 MHz and 400 MHz/100 MHz spectrometer. Conditions are specified for each spectrum (temperature 25 °C unless specified). Chemical shifts are reported in parts per million (ppm) relative to residual solvent and coupling constants (*J*) are reported in hertz (Hz). Signals are described as s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), ddd (doublet of doublet of doublets), qd (quartet of doublets) and br s (broad singlet).

HRMS were obtained on a Q-Tof LC/MC apparatus using electrospray ionization mode and time-of flight analyzer (ESI-TOF).

Melting points were determined in open capillary tubes. Specific rotations were measured

using a 10 cm cell with a Na 589 nm filter.

Methyl ((2-nitrophenyl)sulfonyl)-*L*-phenylalaninate 1. To a solution of methyl *L*-phenylalaninate hydrochloride (2.40 g, 11.1 mmol, 1 equiv) and NEt_3 (4.64 mL, 33.4 mmol, 3 equiv) in CH_2Cl_2 (60 mL) was added 2-nitrobenzenesulfonyl chloride (3.70 g, 16.7 mmol, 1.5 equiv). The mixture was stirred for 3 h at rt. The organic layer was washed with a solution of $NaHCO_3$, water and NaCl. The organic layer was dried on anhydrous Na_2SO_4 and evaporated. The residue was purified on silica gel column eluting with 20-40% EtOAc in *n*-heptane (R_f 0.18, 40% EtOAc in *n*-heptane) to afford the desired product (3.20 g, 8.78 mmol, 79%) as a light yellow oil: $[\propto]_D^{20} = -89.2\,°(c\ 0.19, CDCl_3)$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93-7.90 (m, 1H), 7.81-7.78 (m, 1H), 7.68-7.59 (m, 2H), 7.21-7.04 (m, 5H), 6.02 (d, *J* = 8.7 Hz, 1H), 4.44 (ddd, *J* = 8.7, 7.1, 5.6 Hz, 1H), 3.50 (s, 3H), 3.14 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.04 (dd, *J* = 13.9, 7.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 147.5, 135.0, 134.0, 133.7, 133.1, 130.4, 129.3, 128.7, 127.5, 125.6, 57.9, 52.6, 39.2; HRMS (ESI-TOF) calcd for $C_{16}H_{16}N_2O_6SNa$ $[M+Na]^+$ 387.06214, found 387.06233.

Methyl *N*-((2-nitrophenyl)sulfonyl)-*N*-propyl-*L*-phenylalaninate 2. To a mixture of methyl ((2-nitrophenyl)sulfonyl)-*L*-phenylalaninate (3.00 g, 8.23 mmol, 1 equiv) and K_2CO_3 (2.28 g, 16.5 mmol, 2 equiv) in DMF (40 mL), 1-iodopropane (1.61 mL, 16.5 mmol, 2 equiv) was added. The mixture was stirred for 16 h at rt and evaporated. The residue was purified on silica gel column eluting with 20-40% EtOAc in *n*-heptane (R_f 0.38, 40% EtOAc in *n*-heptane) to afford the desired product (3.12 g, 7.68 mmol, 93%) as a light yellow oil: $[\propto]_D^{20} = -25.8\,°(c\ 0.22, CDCl_3)$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.78 (m, 1H), 6.66-6.51 (m, 3H), 7.28-7.16 (m, 5H), 4.86 (t, *J* = 7.6 Hz, 1H), 3.52 (s, 3H), 3.45-3.32 (m, 2H), 3.26-3.16 (m, 1H), 3.01 (dd, *J* = 14.1, 7.2 Hz, 1H), 1.70-1.52 (m, 2H), 0.86 (t, *J* = 7.4 Hz, 3H); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 170.8, 148.3, 136.6, 133.7, 133.3, 131.6, 130.9, 129.3, 128.7, 127.1, 124.1, 61.4, 52.4, 48.4, 36.9, 23.8, 11.5; HRMS (ESI-TOF) calcd for $C_{19}H_{22}N_2O_6SNa$ $[M+Na]^+$ 429.10908, found 429.11023.

Methyl propyl-*L*-phenylalaninate 3.[20] To a solution of methyl *N*-((2-nitrophenyl)sulfonyl)-*N*-propyl-*L*-phenylalaninate (3.10 g, 7.63 mmol, 1 equiv) and thiophenol (1.17 mL, 11.4 mmol, 1.5 equiv) in DMF (41 mL), K_2CO_3 (2.11 g, 15.3 mmol, 2 equiv) was added. The mixture was stirred at rt for 3 h and evaporated. Purification on silica gel column eluting with 20-40% EtOAc in *n*-heptane (R_f 0.32, 30% EtOAc in *n*-heptane) afforded the desired product (1.69 g, 7.63 mmol, 100%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.15 (m, 5H), 3.66 (s, 3H), 3.54 (t, *J* = 7.0 Hz, 1H), 2.97 (dd, *J* = 7.0, 2.9 Hz, 2H), 2.60-2.53 (m, 1H), 2.49-2.41 (m, 1H), 1.55-1.41 (m, 3H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 137.4, 129.1, 128.4, 126.7, 63.2, 51.6, 50.2, 39.8, 23.2, 11.6; LRMS (ESI-TOF) *m/z* = 222.2 $[M+H]^+$.

(*S*)-5-Benzyl-2-methyl-4-propyl-1,2,4-triazinane-3,6-dione 4. To a solution of triphosgene (270 mg, 0.91 mmol, 0.335 equiv) in anhydrous THF (16.3 mL), under argon, a solution of *N*-propyl *L*-phenylalanine methyl ester (601 mg, 2.72 mmol, 1 equiv) and DIEA (520 μL, 2.99 mmol, 1.1 equiv) in anhydrous THF (6.8 mL) was added dropwise (over 5 minutes). The mixture was stirred at rt for 15 minutes. A solution of *tert*-butyl 2-methylhydrazine-1-carboxylate (397 mg, 2.72 mmol, 1 equiv) and DIEA (520 μL, 2.99 mmol, 1.1 equiv) in anhydrous THF (4.0 mL) was added dropwise. The mixture was heated at 40 °C overnight and evaporated *in vacuo*. The crude was then treated with a solution of TFA/H_2O (95:5, 2 mL) for 1 h at room temperature and concentrated under reduced pressure. Purification on silica gel column eluting with a gradient of 20% EtOAc in *n*-heptane (R_f 0.22) afforded 525

mg (2.01 mmol, 74%) of the title compound as a white solid: mp 140.7-142.7 °C; $[\propto]_D^{20} = +9.8\ (c\ 0.2, \mathrm{MeOH})$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.16 (m, 5H), 4.07-4.01 (m, 1H), 3.82-3.70 (m, 1H), 3.11-2.98 (m, 2H), 2.74 (s, 3H), 2.57-2.47 (m, 2H), 1.61-1.43 (m, 2H), 0.89-0.82 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 153.8, 135.4, 129.9, 128.9, 127.5, 61.8, 48.1, 36.2, 34.8, 21.5, 11.3; HRMS (ESI-TOF) calcd for $C_{14}H_{20}N_3O_2$ $(M+H)^+$ 262.15500, found 262.15485.

Methyl *N*-((*tert*-butoxycarbonyl)-*L*-alanyl)-*N*-propyl-*L*-phenylalaninate 5. (*tert*-Butoxycarbonyl)-*L*-alanine (339 mg, 1.79 mmol, 1 equiv) was dissolved in anhydrous THF (5 mL). 2,4,6-Collidine (954 µL, 7.17 mmol, 4 equiv) and BTC (213 mg, 0.72 mmol, 0.4 equiv) were added to the mixture, which was allowed to stir at rt for 5 min. A solution of methyl propyl-*L*-phenylalaninate (462 mg, 1.79 mmol, 1 equiv) in anhydrous THF (5 mL) was added and the mixture was stirred at rt for 16 h. The mixture was evaporated under reduced pressure. The residue was purified on silica gel eluting with a gradient of 30-60% EtOAc in *n*-heptane followed by a purification on flash chromatography with 5-100% MeCN in water (R_f 0.29, 50% EtOAc in *n*-heptane) to afford the desired product as a white solid (406 mg, 1.03 mmol, 58%): mp 99.8 - 101.8 °C; $[\propto]_D^{20} = -181.4\ °(c\ 0.17, \mathrm{MeOH})$; ^{1}H NMR (500 MHz, DMSO-d_6, 70 °C) δ 7.33-7.13 (m, 5H), 6.44 (br s, 1H), 4.36-4.21 (m, 2H), 3.59 (s, 3H), 3.25 (dd, J = 14.0, 5.0 Hz, 1H), 3.17-3.00 (m, 2H), 2.72-2.62 (m, 1H), 1.45-1.34 (m, 11H), 1.11 (d, J = 6.9 Hz, 3H), 0.74 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.6, 170.8, 154.8, 138.1, 129.3, 128.1, 126.4, 77.8, 61.6, 51.5, 51.1, 46.1, 34.0, 28.1, 21.3, 17.9, 10.8; HRMS (ESI-TOF) calcd for $C_{21}H_{32}N_2O_5Na$ $(M+Na)^+$ 415.22035, found 415.22041.

(*S*)-1-(((*S*)-1-Methoxy-1-oxo-3-phenylpropan-2-yl)(propyl)amino)-1-oxopropan-2-aminium 2,2,2-trifluoroacetate 6. Methyl *N*-((*tert*-butoxycarbonyl)-*L*-alanyl)-*N*-propyl-*L*-

phenylalaninate (373 mg, 0.95 mmol, 1 equiv) was solubilized in a mixture of trifluoroacetic acid and water (2.1 mL, 95:5). The mixture was allowed to stir at rt at 0 °C for 1 h and was then evaporated to a slurry (386 mg, 0.95 mmol, 100%). Analysis of the crude product by NMR showed only trifluoroacetate **6** without product from cyclisation. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (br s, 3H), 7.26-6.96 (m, 5H), 4.31-4.20 (m, 1H), 3.86 (dd, *J* = 10.9, 4.8 Hz, 1H), 3.60 (s, 3H), 3.31-3.12 (m, 2H), 2.93-2.81 (m, 1H), 2.37-2.25 (m, 1H), 1.42-1.26 (m, 5H), 0.67 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.6, 161.2 (q, *J* = 38 Hz), 136.9, 129.3, 129.0, 127.5, 115.5 (q, *J* = 288 Hz), 63.6, 52.9, 52.5, 48.0, 34.3, 21.5, 17.0, 10.6; HRMS (ESI-TOF) calcd for $C_{16}H_{25}N_2O_3$ $(M+H)^+$ 293.186518, found 293.186546.

(3*S*,6*S*)-6-Benzyl-3-methyl-1-propylpiperazine-2,5-dione 7.[20] (*S*)-1-(((S)-1-Methoxy-1-oxo-3-phenylpropan-2-yl)(propyl)amino)-1-oxopropan-2-aminium 2,2,2-trifluoroacetate **4** (227 mg, 0.68 mmol, 1 equiv) and Et_3N (380 µL, 2.74 mmol, 4 equiv) were dissolved in DMF (5 mL). The mixture warmed to 60 °C and stirred overnight. The mixture was then evaporated to a slurry. The residue was purified on flash chromatography eluting with 5-100% MeCN in water (R_f 0.34, 5% MeOH/CH_2Cl_2) to afford 135 mg (0.52 mmol, 76%) of the desired product as a white solid: mp 218.3–220.3 °C, lit 221 - 223 °C; $[\propto]_D^{20} = +56.0$ (c 0.50, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (br s, 1H), 7.33-7.20 (m, 3H), 7.07-7.02 (m, 2H), 4.21 (t, *J* = 4.2 Hz, 1H), 3.84 (ddd, *J* = 13.4, 9.2, 6.3 Hz, 1H), 3.64 (qd, *J* = 6.9 Hz, 2.8 Hz, 1H), 3.17 (dd, *J* = 13.9, 4.8 Hz, 1H, AB system), 3.08 (dd, *J* = 13.9 Hz, 3.6 Hz, 1H, AB system), 2.90 (ddd, *J* = 13.4, 9.0, 5.5 Hz, 1H), 1.67-1.44 (m, 2H), 0.84 (t, *J* = 7.4 Hz, 3H), 0.26 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 165.2, 135.6, 130.2, 128.3, 126.9, 59.8, 50.0, 44.8, 36.2, 20.0, 19.5, 11.1; HRMS (ESI-TOF) calcd for $C_{15}H_{21}N_2O_2$ $(M+H)^+$ 261.15975, found 261.15949.

Solubility. Thermodynamic solubility was measured by dissolving compounds **4** or **7** up to saturation in a pH 7.4 phosphate-buffered saline (PBS) with the following composition: 137.5 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, and 1.4 mM KH_2PO_4. Samples were shaken for 24 h at 20 °C. Saturation was confirmed by the presence of undissolved powder. After ultracentrifugation, the concentration in the supernatant was measured by an HPLC procedure using a calibration curve established for each compound by diluting a 10 mM DMSO stock solution to adapted concentrations.

Stability in mouse plasma. Stability was determined in mouse plasma at 37 °C after 6 h. For each compound, the 10 mM DMSO stock solution was diluted in MeCN to a final concentration of 100 μM. This solution was again diluted in plasma to a final concentration of 1 μM. The mixture was divided into two aliquots. The incubation of each aliquot was stopped at t_0 and 6 h by adding one volume of ice cold acetonitrile. Samples were stirred for 3 min, sonicated for 3 min, and then centrifuged at 4 °C before HPLC injection. The percentage of remaining test compound relative to t_0 was measured by monitoring the peak area on the chromatogram.

Microsomal stability in mouse hepatocytes. Microsomal stability was determined in mouse hepatocytes at 37 °C after 30 min. For each compound, the 10 mM DMSO stock solution was diluted in DMSO to a final concentration of 100 μM. This solution was again diluted to a final concentration of 1 μM in pH 7.4 phosphate-buffered saline (PBS) containing mouse liver microsomes (0.5 mg/mL), 1 mM of NaDPH, and 3 mM of $MgCl_2$. The mixture was divided into two aliquots. The incubation of each aliquot was stopped at t_0 and 30 min by adding one volume of ice-cold acetonitrile. Samples were stirred for 3 min, sonicated for 3 min, and then

centrifuged at 4 °C before HPLC injection. The percentage of remaining test compound relative to t_0 was measured by monitoring the peak area on the chromatogram.

ACKNOWLEDGMENTS

This work was supported by the Centre National de la Recherche Scientifique, the Université de Strasbourg (UDS) and the LabEX Medalis (ANR-10-LABX-0034). We are grateful to Barbara Lamure and Justine Viéville for NMR experiments and Patrick Wehrung for MS analyses (Service Commun d'Analyse, Unistra).

SUPPORTING INFORMATION

^{1}H NMR and ^{13}C NMR spectra for all compounds, X-ray crystal structures and crystallographic data for **4** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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