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PAPER

Stereoselective synthesis and rearrangement-fragmentation of arylidene *N*-alkoxydiketopiperazines†

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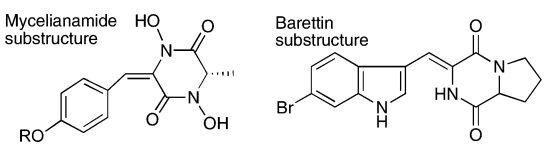
A stereoselective synthesis of arylidene *N*-alkoxydiketopiperazines *via* oxime-ether formation and intramolecular acylation is described, followed by an acid-catalysed rearrangement-fragmentation to give novel diketopiperazine hemiaminal derivatives with useful bioactivity against certain tumour cell lines.

Introduction

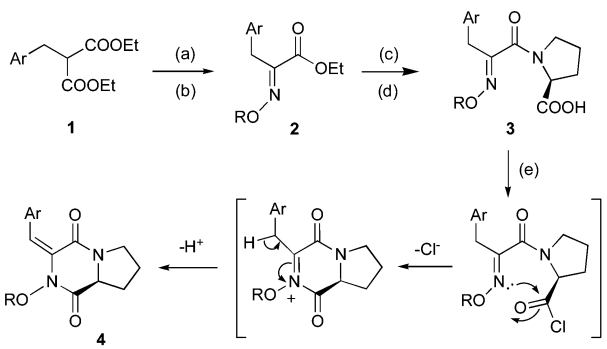
The diketopiperazine (DKP) group is widespread in natural products isolated from microorganisms, sponges, tissues and body fluids,[1,2] of which, the 3-ylidene-DKPs are one such example. Many DKPs exhibit remarkable biological and pharmacological activities, *e.g.* antimicrobial, antitumor, antiviral and an ability to regulate plant growth.[3] Thus, mycelianamide, an arylidene-*N*-hydroxyDKP isolated from the mycelium of *Penicillium griseofulvum*,[4] is noted for its unique bis-hydroxamic acid sub-structure and its inhibition of the growth activity of a number of Gram-positive bacteria *in vitro*. Barettin, which was isolated from cold water marine sponge *Geodia Barettin*, also belongs to this class of arylidene-DKPs and exhibits antibacterial and antiviral activities, and inhibits settling barnacle larvae.[5]



Although DKPs can be formed by cyclization of dipeptides, such reactions are rarely used to prepare arylidene-DKPs due to the instability of the enamine intermediate and poor stereoselectivity. For example, mycelianamide was first synthesised by Cava and Hinmo[6] as a racemate; the *Z*- and *E*-isomers were formed without stereoselectivity and proved difficult to separate. The arylidene double bond geometry of barettin had been debated for several years; it was eventually confirmed by total synthesis to be *Z*, although the arylidene intermediate was a mixture of *E*- and *Z*-isomers.[7] Liebscher *at al*. have reviewed the synthesis, properties and applications of 3-ylidene-DKPs[8] and other methods of synthesis were also reported.[9] Hence, the development of new stereoselective synthetic routes to arylidene *N*-alkoxyDKPs is a useful synthetic goal. Herein, we report a novel, efficient and stereoselective synthesis of *N*-alkoxy derivatives of *E*-arylidene bicyclo-DKPs, together with an unusual fragmentation of an arylidene-*N*-alkoxyDKP on the way to a DKP-containing hemiaminal.

Results and discussion

As outlined in Scheme 1, this approach starts from *N*-(2-alkoxyimino-3-arylpropinoyl)-proline **3**, which was prepared by the reaction of L-proline methyl ester with (2-*O*-alkyloxime-1-aryl)propionic acid in the presence of HBTU. Compound **3** was treated with $SOCl_2$ to produce the corresponding acyl chloride, which directly underwent intramolecular *N*-acylation with the oxime ether to stereoselectively generate compounds **4** in good overall yields (Table 1). The reaction route is likely to involve deprotonation of the cyclic *N*-acylation intermediate.



Reagents: (a) EtONO, EtONa; (b) RBr, K_2CO_3; (c) (i) NaOH, H_2O, EtOH, then H_3O^+; (ii) HBTU, DMF, L-proline methyl ester.HCl; (d) LiOH, THF, H_2O; (e) $SOCl_2$, CH_2Cl_2.

Scheme 1 Synthesis of *E*-arylidene diketopiperazines **4**.

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Table 1 Structures and yields for compounds **4**

Product	Ar	R	Yield (%)	Arylidine C=C**H** δ, ppm
4a	3-pyridyl	*c*-hexyl	88	7.05
4b	Ph	*c*-hexyl	85	7.12
4c	Ph	Me	87	7.21
4d	Ph	*n*-Bu	80	7.18
4e	Ph	benzyl	88	7.24
4f	3,4,5-tri-(methoxy)-phenyl	*c*-hexyl	83	7.04
4g		benzyl	87	7.16
4h		*n*-Bu	85	7.10

The structure of compound **4a**, with its *E*-configuration of the exocyclic C(1)=C(14) bond (Fig. 1) was confirmed by single crystal X-ray diffraction.‡ In addition, similar chemical shifts of the H(14) singlet (δ in Table 1) suggest the same configuration for other compounds **4**. Although **4a** formed chiral crystals, its absolute configuration at C(6) was not determined crystallographically. However, chiral HPLC of **4c** (see ESI†) showed that the (*S*)-configuration of **3** had been retained with 99.3% e.e.

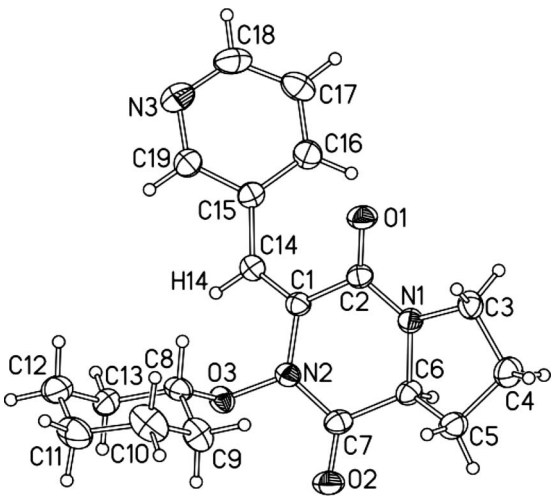
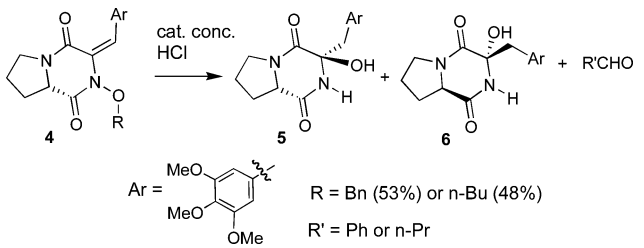


Fig. 1 X-ray molecular structure of **4a**, showing 30% thermal ellipsoids. (*S*)-configuration at C(6) was assigned on the basis of chiral HPLC of **4c**.

Arylidene *N*-alkoxydiketopiperazines **4** have not been reported much to date and hence it was necessary to study their chemical characteristics. Compounds **4g** and **4h** were found to be particularly unstable when exposed to catalytic HCl (Scheme 2), being converted to the corresponding enantiomeric cyclic aminals **5** and **6** with concomitant release of an aldehyde by-product.

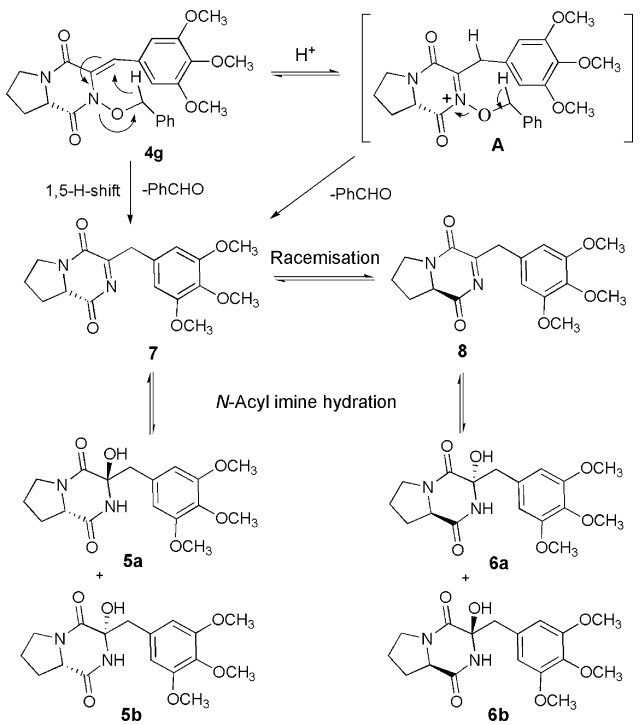
Interestingly, this reaction (Scheme 2) was found to be highly dependent upon the substituent characteristics, and hence did

‡ CCD area detector, Mo-Kα radiation (λ = 0.71073 Å), T = 294 K. *Crystal data*: **4a**, $C_{19}H_{23}N_3O_3$, M = 341.40, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 9.7941(14), b = 10.3009(15), c = 17.223(2) Å, U = 1737.6(4) Å^3, Z = 4, 9847 reflections with 2$\theta \leq 53°$ (2047 unique after merging 1520 Friedels), R_{int} = 0.033, R = 0.032 [$I \geq 2\sigma(I)$], w$R(F^2)$ = 0.083. CCDC-696110. **5a·6a**, $C_{17}H_{22}N_2O_6$, M = 350.37, triclinic, space group $P\bar{1}$(no. 2), a = 7.525(3), b = 10.852(4), c = 11.633(4) Å, α = 103.951(6), β = 105.568(7), γ = 99.030(6)°, U = 862.8(6) Å^3, Z = 2, 4282 reflections with 2$\theta \leq 50°$ (3014 unique), R_{int} = 0.025, R = 0.058 [$I \geq 2\sigma(I)$], w$R(F^2)$ = 0.191. CCDC-696109.



Scheme 2 Hydrolysis of compounds **4g,h** with electron rich Ar groups.

not occur with the electron deficient pyridine-containing system **4a** or even the less electron deficient phenyl systems **4b–4e**. A likely explanation is that when the aryl is trimethoxyphenyl (*i.e.* **4g** and **h**), either a facile 1,5-hydrogen shift occurs or, considering the acidity of the reaction mixture, that protonation of the exocyclic double bond occurs (to give species **A**) to give an intermediate *N*-acyl imine **7** (Scheme 3, using **4g** as the example). However, when the aryl is phenyl or pyridyl, this rearrangement (or protonation) is less facile and fails to occur. Following the plausible mechanism for the fragmentation-racemisation reaction shown in Scheme 3, the reason for the isolation (by crystallisation directly from the reaction mixture) of the racemic mixture[8] of **5a** and **6a** becomes apparent, especially after NMR studies (*vide infra*). The intermediate formation of the *N*-acyl imine **7** renders the proline derived chiral centre C–H highly susceptible to enolisation and hence racemisation occurs readily, resulting in equilibration between **7** and its enantiomer **8**. Both *N*-acyl imines **7** and **8** will undergo facile and reversible acyl imine hydration, resulting in a mixture of isomeric *N*-acyl hemiaminals **5a/b** and **6a/b**, as also outlined in Scheme 3. A racemic single crystal of the **5a·6a** enantiomeric pair was subsequently obtained and studied by X-ray diffraction (see ESI†).



Scheme 3 Possible mechanism for the rearrangement-fragmentation reaction.

Table 2 *In vitro* % inhibition data of compounds **4a–4h** (20 μg mL^{-1}) toward K562-1, HCT-15, HT-29, HCT-8, HePG2, MDA-MB-231, A-549 cell lines

Compound	K562-1	HCT-15	HT-29	HCT-8	HePG2	MDA-MB-231	A549
4a	69.3	62.1	70.3	80.1	60.4	16.1	35.2
4b	78.2	72.3	69.4	76.3	68.5	17.3	33.8
4c	90.4	88.8	65.4	78.8	62.2	23.5	26.6
4d	96.2	90.6	64.9	68.4	59.9	16.3	50.8
4e	65.6	69.7	76.2	66.8	69.2	32.6	41.3
4f	80.1	67.9	60.5	75.2	76.1	42.5	26.4
4g	73.2	74.2	70.1	79.1	75.0	43.2	38.9
4h	64.4	61.6	61.6	60.6	64.3	45.6	43.4

In support of the equilibrium process described in Scheme 3, ^{1}H NMR spectra of a crystal of a 1 : 1 mixture of enantiomers **5a** and **6a** in $CDCl_3$ shows all the proton signals doubling up into pairs with a ratio of about 1 : 1 for each set of peaks, *i.e.* indicative of diastereoisomeric pairs, and no longer existing as identical enantiomeric pairs. In addition, examination of the ^{1}H NMR spectra of the mother liquors from the crystallisation reaction mixture showed that a diastereoisomeric ratio of 3 : 1 was present. The difference between these two results is readily explained by the fact that under strongly acidic conditions, the equilibrium ratio of the mixture of diastereoisomeric pairs (3 : 1) is different to that which occurs under neutral conditions (1 : 1). Hence, the fragmentation-racemisation reaction results in a dynamic stereoisomeric mixture of compounds **5a/b** and **6a/b** from which a 1 : 1 mixture of **5a**:**6a** crystalises out, presumably being particularly stable in the solid state. Once back in solution, whether under acidic or neutral conditions, the mixture of isomers exists in equilibrium with facile interconversion.

In addition to examining their chemical properties, eight of the *E*-arylidene *N*-alkoxyDKPs (*i.e.* **4a–4h**) were tested for their ability to inhibit tumor cells, including the cell lines K562-1, HCT-15, HT-29, HCT-8, HePG2, MDA-MB-231 and A-549 (Table 2). The results showed that all of the diketopiperazines exhibited above 60% inhibition at concentrations of 20 μg mL^{-1} against cell lines K562-1, HCT-15, HT-29, HCT-8 and HePG2. From the results, we can see that the cytostatic activities of compounds **4c** and **4d** are much higher than the other systems, and hence further evaluation of their bioactivities showed that the IC_{50} values of **4c** and **4d** against cell lines K562-1 were found to be 18 μM and 27 μM respectively, and against cell line HCT-15 were found to be 14 μM and 12 μM, respectively, confirming the interesting antitumor potential of these types of compounds.[3]

Summary and conclusions

In summary, a stereoselective route has been developed for the synthesis of *E*-arylidene *N*-alkoxydiketopiperazines using *N*-(2-alkoxylimino-3-arylpropinoyl)proline derivatives **3** as the key intermediates in yields above 80%. Subsequently, a novel rearrangement-fragmentation reaction of arylidene *N*-alkoxyDKPs was discovered in the case of an electron rich aryl substituent such as trimethoxyphenyl. It is proposed that this rearrangement-fragmentation derives a new cyclic *N*-acyl imine species which rapidly racemises and hydrates to give novel diketopiperazines containing a hemiaminal function, the structure of one of which was confirmed by X-ray diffraction studies. These types of DKPs also showed interesting antitumour activities in certain cases; further studies in this direction are underway.

Experimental

General experimental

^{1}H NMR and ^{13}C spectra were recorded on a Bruker Advance II 500 instrument in $CDCl_3$ solution, using tetramethylsilane as an internal reference, operated at 500 for ^{1}H and 126 MHz for ^{13}C. Elemental analyses were performed on a Perkin-Elmer 2400 C instrument. Infrared spectra were recorded on a Shimadzu Bio-Rad FTS 135 instrument. HPLC analyses was performed on a Dionex's UltiMate-3000 HPLC, 10 μM, 150 × 2.1 mm Daicel Chiralcel OD column with a flow rate of 0.5 mL min^{-1} using the mixture of 85% water (0.15% formic acid) with 15% MeOH.

Most reagents were commercially available reagent grade chemicals and were used without further purification, unless noted otherwise. Starting material diethyl arylmethylene malonate **1** was synthesized using a published method.[10] *N*,*N*-Dimethylformamide (DMF) was dried over CaH_2 for 2 days and then distilled under a reduced pressure prior to use. Ethanol was refluxed over sodium turnings and then distilled fractionally. Flash chromatography was performed with silica gel (200-400 mesh).

General procedure for the preparation of ethyl 2-alkyloxyimine-3-arylpropionate 2

Diethyl arylmethylmalonate (0.10 mol) was added to a 250 ml reaction flask, which was fitted with mechanical stirrer, reflux condenser with drying tube and dropping funnel. After cooling to −15 °C, ethyl nitrite (0.105 mol) was added to the reaction solution. Then EtONa/EtOH solution (0.10 mol) was slowly added dropwise with mechanical stirring. The mixture was placed in a refrigerator for 12 h. The solution was concentrated, the residue obtained was added to an equal volume of water. The pH of the mixture was adjusted to 6 with dilute hydrochloric acid, extracted with ethyl acetate (4 × 50 ml), and dried over anhydrous Na_2SO_4. After the solvent was removed under reduced pressure, the ethyl 2-hyroxyimine-3-arylpropionate crude product was obtained and used for the next reaction without purification (yield >90%). The crude product (0.05 mol), 50 ml acetone and anhydrous K_2CO_3 (0.055 mol) were added to a 250 ml reaction flask, which was fitted with a mechanical stirrer, reflux condenser with dry tube and dripping funnel. Then alkyl chloride or alkyl bromide (0.10 mol) was added dropwise to the reaction solution. The mixture was stirred 4 h at 35–40 °C. After the precipitate was filtered, the solution was concentrated to give the residue, which

was purified by silica gel column chromatography to obtain the compounds **2** predominantly as syrups unless otherwise stated.

Ethyl 2-cyclohexyloxyimine-3-β-pyridylpropionate 2a

The product **2a** was obtained according to the general procedure in 89% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 1). IR (KBr) *ν* 1714, 1574, 1478 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.20–1.23 (m, 1H), 1.29–1.31 (m, 2H), 1.40–1.43 (m, 3H), 1.67–1.69 (m, 2H), 1.95–1.98 (m, 2H), 3.83 (s, 2H), 3.85–3.88 (m, 1H), 4.24 (q, *J* 7.0 Hz, 2H), 7.12 (d, *J* 5.0 Hz, 1H), 7.53 (d, *J* 9.0 Hz, 1H), 8.36 (d, *J* 5.0 Hz, 1H), 8.48 (d, *J* 2.0 Hz, 1H) ppm; Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65; Found: C, 66.23; H, 7.66; N, 9.61.

Ethyl 2-cyclohexyloxyimine-3-phenylpropionate 2b

The product **2b** was obtained according to the general procedure in 85% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν* 1715, 1574, 1496 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.20–1.22 (m, 2H), 1.29 (t, *J* 7.0 Hz, 3H), 1.32–1.35 (m, 1H), 1.60–1.64 (m, 1H), 1.84–1.97 (m, 6H), 4.07 (s, 2H), 4.28 (q, *J* 7.0 Hz, 2H), 5.21–5.24 (m, 1H), 7.15–7.18 (m, 2H), 7.24–7.27 (m, 3H) ppm; Anal. Calcd for $C_{17}H_{23}NO_3$: C, 70.56; H, 8.01; N, 4.84; Found: C, 70.53; H, 8.05; N, 4.88.

Ethyl 2-methoxyimine-3-phenylpropionate 2c

The product **2c** was obtained according to the general procedure in 90% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν*: 1713, 1570, 1493 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.30 (t, *J* 7.0 Hz, 3H), 3.93 (s, 2H), 4.09 (s, 3H), 4.28 (q, *J* 7.0 Hz, 2H), 7.18–7.21 (m, 2H), 7.24–7.26 (m, 3H) ppm; Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33; Found: C, 65.12; H, 6.85; N, 6.31.

Ethyl 2-butyloxy imine-3-phenylpropionate 2d

The product **2d** was obtained according to the general procedure in 87% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν* 1717, 1578, 1500 cm^{-1};^1H NMR (500 MHz, $CDCl_3$) 0.91 (t, *J* 7.5 Hz, 3H), 1.25 (t, *J* 7.5 Hz, 3H), 1.31–1.35 (m, 2H), 1.65–1.68 (m, 2H), 3.92 (s, 2H), 4.23 (q, *J* 7.5 Hz, 2H), 4.28 (q, *J* 7.5 Hz, 2H), 7.14–7.16 (m, 2H), 7.23–7.25 (m, 3H) ppm; Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32; Found: C, 68.46; H, 8.02; N, 5.29.

Ethyl 2-benzyloxyimine-3-phenylpropionate 2e

The product **2e** was obtained according to the general procedure in 92% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν* 1718, 1580, 1500, 1468 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.31 (t, *J* 7.0 Hz, 3H), 3.95 (s, 2H), 4.29 (q, *J* 7.0 Hz, 2H), 5.33 (s, 2H), 7.20–7.25 (m, 5H), 7.31–7.35 (m, 5H) ppm; Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71; Found: C, 72.68; H, 6.47; N, 4.74.

Ethyl 2-cyclohexyloxyimine-3-(3,4,5-trimethoxy)phenylpropionate 2f

The product **2f** was obtained according to the general procedure in 83% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 5). IR (KBr) *ν* 1718, 1588, 1566, 1484 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.27– 1.29 (m, 1H), 1.30–1.33 (m, 3H), 1.35–1.37 (m, 2H), 1.51–1.54 (m, 3H), 1.71–1.74 (m, 2H), 1.97–2.00 (m, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 3.88 (s, 2H), 4.35–4.37 (m, 1H), 6.54 (s, 2H) ppm; Anal. Calcd for $C_{20}H_{29}NO_6$: C, 63.31; H, 7.70; N,3.69; Found: C, 63.33; H, 7.69; N, 3.71.

Ethyl 2-benzyloxyimine-3-(3,4,5-trimethoxy)phenyl propionate 2g

The product **2g** was obtained according to the general procedure in 87% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 5). IR (KBr) *ν* 1719, 1592, 1569, 1484 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.33 (t, *J* 7.0 Hz, 3H), 3.68 (s, 3H), 3.79 (s, 3H), 3.84 (s, 3H), 3.87 (s, 2H), 4.30 (q, *J* 7.0 Hz, 2H), 5.34 (s, 2H), 6.46 (s, 2H), 7.31–7.35 (m, 5H) ppm; Anal. Calcd for $C_{21}H_{25}NO_6$: C, 65.10; H, 6.50; N, 3.62; Found: C, 65.11; H, 6.47; N, 3.59.

Ethyl 2-butyloxyimine-3-(3,4,5-trimethoxy)phenyl propionate 2h

The product **2h** was obtained according to the general procedure in 90% after purification by silica gel column chromatography (EtOAc–hexane, 1 : 5). IR (KBr) *ν* 1719, 1592, 1570, 1482 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.94 (t, *J* 7.5 Hz, 3H), 1.33 (t, *J* 7.0 Hz, 3H), 1.40–1.42 (m, 2H), 1.69–1.72 (m, 2H), 3.80 (s, 3H), 3.83 (s, 6H), 3.87 (s, 2H), 4.29–4.32 (m, 4H), 6.51 (s, 2H) ppm; Anal. Calcd for $C_{18}H_{27}NO_6$: C, 61.17; H, 7.70; N, 3.96; Found: C, 61.13; H, 7.72; N, 4.05.

General procedure for the preparation of *N*-(2-alkyloxyimine-3-arylpropionyl)proline 3

To a 100 ml reaction flask with mechanical stirrer and reflux condenser thermometer the above preparation product **2** (0.02 mol) and 60 ml 2*N* NaOH, were added. The mixture was stirred 2 h at 95 °C. After cooling, the solution was acidified with hydrochloric acid to pH 3. Then the mixture was extracted with EtOAc (5 × 30 ml) and the extract was dried over $MgSO_4$. The solution was concentrated under reduced pressure to give the corresponding crude product carboxylic acid, which was used in the next reaction without purification. $(C_2H_5)_3N$ (1.5 ml) and HBTU (2.6 g) were added to 30 ml DMF solution of 6.5 mmol of the above corresponding product under stirring, then methyl proline hydrochloride salt (6.8 mmol) was added dropwise. The mixture was stirred 24 h at 35–40 °C, then 10 ml H_2O was added. The pH of the mixture was adjusted to 6 with diluted hydrochloric acid, and extracted with CH_2Cl_2 (2 × 50 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to give a solid residue. The crude product was purified by flash chromatography on silica gel to give the coupled products. To a 100 ml reaction flask with 0.1 mol of the above reaction products and solvent (THF–H_2O = 7 : 1) 5 eq LiOH was added with stirring. The mixture was stirred 12 h at room temperature. The solution was concentrated, then an equal volume water was added. The pH

of the solution was adjusted to 3.0 with hydrochloric acid, and extracted with ethyl acetate (30 ml × 5). The organic phase was dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give the crude products **3** (**3a–3g** and **3c**, racemic) (yield >95%) (predominantly as syrups unless otherwise stated), which were used in the next step without further purification.

General procedure the preparation of *N*-alkoxydiketopiperazines 4

To 100 ml reaction flask 0.1 mol compound **3a–h** and 40 ml anhydrous benzene were added and cooled to 0 °C. 0.15 ml $SOCl_2$ was added dropwise with stirring. The reaction was continued for 4 h at room temperature. The solution was concentrated *in vacuo* to give a syrupy residue, then an equal volume of water was added with stirring. The pH of the solution was adjusted to 8 with hydrochloric acid, extracted with CH_2Cl_2 (30 ml × 5). The organic phase was dried over anhydrous $MgSO_4$, filtered, concentrated under reduced pressure to give a crude product, which was purified by silica gel flash chromatography to give the title compounds **4**, predominantly as syrups unless otherwise stated.

Cyclo-[*N*-cyclohexyloxy-2-[(β-pyridyl)-methylene]glycyl-prolyl] 4a

The product **4a** was obtained as a white solid according to the general procedure in 88% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). Crystals suitable for single crystal X-ray analysis were grown by slow evaporation from $CHCl_3$. Mp 129.5~131 °C; IR (KBr) *ν* 1712, 1666, 1631, 1586, 1480 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.90–1.16 (m, 6H), 1.38–1.41 (m, 1H), 1.47–1.49 (m, 1H), 1.59–1.62 (m, 1H), 1.67–1.69 (m, 1H), 1.97–2.02 (q, *J* 8.5 Hz, 1H), 2.09–2.17 (m, 2H), 2.50–2.54 (m, 1H), 3.66–3.76 (m, 4H), 4.30 (q, *J* 7.0 Hz, 1H), 7.05 (s, 1H), 7.76 (d, *J* 9.0 Hz, 1H), 8.50 (d, *J* 5.0 Hz, 1H), 8.65 (s, 1H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) 162.1, 157.8, 151.9, 150.1, 137.0, 128.6, 128.3, 121.4, 114.0, 81.9, 57.5, 44.5, 29.0, 28.9, 27.7, 24.1, 22.8, 22.6, 20.0; Anal. Calcd for $C_{19}H_{23}N_3O_3$: C, 66.84; H, 6.79; N, 12.31; Found: C, 66.88; H, 6.85; N, 12.33.

Cyclo[*N*-cyclohexyloxy-2-(phenylmethylene)glycyl-prolyl] 4b

The product **4b** was obtained according to the general procedure in 85% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν* 1701, 1670, 1624, 1585, 1503 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.88–1.08 (m, 5H), 1.18–1.20 (m, 1H), 1.36–1.39 (m, 1H), 1.46–1.47 (m, 1H), 1.63–1.66 (m, 2H), 1.97–2.00 (m, 1H), 2.10–2.15 (m, 2H), 2.59–2.51 (m, 1H), 3.63–3.74 (m, 3H), 4.29 (q, *J* 6.5 Hz, 1H), 7.12 (s, 1H), 7.28–7.33 (m, 3H), 7.43 (d, *J* 7.0 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): 163.2, 159.6, 132.7, 130.9(2), 128.4, 127.4(2), 119.8, 82.2, 58.4, 45.4, 30.0, 29.5, 28.7, 25.2, 23.8, 23.6, 22.6, 22.4; Anal. Calcd for $C_{20}H_{24}N_2O_3$: C, 70.56; H, 7.11; N, 8.23; Found: C, 70.59; H, 7.15; N, 8.26.

Cyclo[*N*-methyloxy-2-(phenylmethylene)glycyl-prolyl] 4c

The product **4c** was obtained according to the general procedure in 87% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). Chiral HPLC analyses of the product showed a major peak for the (*S*)-enantiomer at R_t 14.5 min (99.3% *e.e.*) compared to racemic **4c** (synthesized by using racemic **3c**) which showed two equal peaks with R_ts of 14.5 min and 15.5 min for the (*S*)- and (*R*)-enantiomers respectively. IR (KBr) *ν* 1698, 1674, 1622, 1578, 1506 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.85–2.02 (m, 1H), 2.11–2.21 (m, 2H), 2.45–2.49 (m, 1H), 3.39 (s, 3H), 3.67–3.72 (m, 2H), 4.29 (q, *J* 7.0 Hz, 1H), 7.21 (s, 1H), 7.31–7.33 (m, 3H), 7.45 (d, *J* 7.0 Hz, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) 162.1, 159.4, 132.5, 130.7 (2), 128.6, 127.6 (2), 126.0, 120.3, 61.5, 58.1, 45.6, 28.2, 22.4; Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29; Found: C,66.20; H, 5.97; N, 10.33.

Cyclo[*N*-butyloxy-2-(phenylmethylene)glycyl-prolyl] 4d

The product **4d** was obtained according to the general procedure in 80% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν* 1705, 1680, 1618, 1577, 1503 cm^{-1}; ^1H-NMR (500 MHz, CDCl3) 0.65 (t, *J* 7.0 Hz, 3H), 0.91–0.95 (m, 2H), 1.03–1.13 (m, 2H), 1.96–1.99 (m, 1H), 2.10–2.18 (m, 2H), 2.46–2.48 (m, 1H), 3.49 (q, *J* 7.0 Hz, 1H), 3.66–3.74 (m, 3H), 4.29 (q, *J* 6.5 Hz, 1H), 7.18 (s, 1H), 7.28–7.33 (m, 3H), 7.44 (d, *J* 7.0 Hz, 2H) ppm; ^{13}C NMR (125 MHz, CDCl3) 162.2, 159.3, 132.8, 130.7(2), 128.5, 127.5(2), 126.6, 120.0, 74.1, 58.1, 45.6, 29.0, 28.3, 22.4, 18.5, 13.6; Anal. Calcd for $C_{18}H_{22}N_2O_3$: C, 68.77; H, 7.05; N, 8.91; Found: C,68.81; H, 7.09; N, 8.94.

Cyclo[*N*-benzyloxy-2-(phenylmethylene)glycyl-prolyl] 4e

The product **4e** was obtained according to the general procedure in 88% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν* 1706, 1681, 1618, 1589, 1577, 1511, 1503 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.92–2.08 (m, 3H), 2.39–2.44 (m, 1H), 3.63–3.66 (m, 2H), 4.26 (q, *J* 6.5 Hz, 1H), 4.56 (s, 2H), 6.83–6.85 (m, 2H), 7.14–7.17 (m, 2H), 7.23–7.25 (m, 2H), 7.30–7.32 (m, 3H), 7.48–7.49 (m, 2H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) 162.4, 159.2, 132.7, 132.5, 131.0(2), 130.1(2), 128.9, 128.7, 128.1(2), 127.7(2), 126.5, 120.4, 75.8, 58.0, 45.6, 29.5, 22.4; Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04; Found: C,72.44; H, 5.82; N, 8.08.

Cyclo[*N*-cyclohexyloxy-2-[(3,4,5-trimethyloxy) phenylmethylene] glycyl-prolyl] 4f

The product **4f** was obtained according to the general procedure in 83% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). IR (KBr) *ν* 1693, 1678, 1621, 1583, 1503,1099 cm^{-1}; ^1H-NMR (500 MHz, CDCl3): 1.01–1.14 (m, 4H), 1.19 (br, 1H), 1.28–1.29 (m, 2H), 1.42 (br, 1H), 1.65–1.72 (m, 2H), 1.99–2.01 (m, 1H), 2.13–2.19 (m, 2H), 2.49–2.51 (m, 1H), 3.69–3.77 (m, 3H), 3.86–3.90 (m, 9H), 4.29 (q, *J* 7.0 Hz, 1H), 6.77 (s, 2H), 7.04 (s, 1H) ppm; ^{13}C NMR (125 MHz, CDCl3) 163.2, 159.9, 152.2, 138.8, 127.9, 126.9, 119.9, 108.5(2), 82.28, 65.9, 61.0, 58.4, 56.3(2), 45.5, 30.8, 30.1, 28.6, 25.2, 23.8, 23.6, 22.5; Anal. Calcd for $C_{23}H_{30}N_2O_6$: C, 64.17; H, 7.02; N, 6.51; Found: C, 64.21; H, 7.07; N, 6.48.

Cyclo[*N*-benzyloxy-2-[(3,4,5-trimethyloxy)phenylmethylene] glycyl-prolyl] 4g

The product **4g** was obtained according to the general procedure in 87% yield after purification by silica gel flash chromatography (EtOAc/hexane, 1 : 2). IR (KBr) *ν* 1698, 1677, 1621, 1608, 1583,

1503, 1099 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.67 (s, 2H), 1.95–1.98 (m, 1H), 2.05–2.09 (m, 2H), 2.41–2.43 (m, 1H), 3.75 (s, 6H), 3.87(s, 3H), 4.25–4.28 (m, 1H), 4.58 (d, *J* 9.0 Hz, 1H), 4.68 (d, *J* 9.0 Hz, 1H), 6.77 (s, 2H), 6.95 (d, *J* 7.5 Hz, 2H), 7.16 (s, 1H), 7.19–7.26 (m, 3H) ppm; Anal. Calcd for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39; Found: C, 65.75; H, 6.01; N, 6.42.

Cyclo[*N*-butyloxy-2-[(3,4,5-trimethyloxy)phenylmethylene]glycyl-prolyl] 4h

The product **4h** was obtained as a white solid according to the general procedure in 85% yield after purification by silica gel flash chromatography (EtOAc–hexane, 1 : 2). Mp. 138.5~141.5 °C; IR (KBr) *v* 1701, 1676, 1608, 1583, 1099 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 0.71 (t, *J* 7.0 Hz, 3H), 0.96–1.05 (m, 2H), 1.19–1.28 (m, 2H), 1.99–2.05 (m, 1H), 2.11–2.18 (m, 2H), 2.44–2.47 (m, 1H), 3.55 (dd, *J* 7.0 and *J* 7.5 Hz, 1H), 3.67–3.70 (m, 2H), 3.77–3.80 (m, 1H), 3.85 (s, 9H), 4.27–4.31 (m, 1H), 6.69 (s, 2H), 7.10 (s, 1H) ppm; Anal. Calcd for $C_{21}H_{28}N_2O_6$: C, 62.36; H, 6.98; N, 6.93; Found: C, 62.39; H, 7.02; N, 6.98.

Preparation of cyclo[2-hydroxy-2-[(3,4,5-trimethyloxy) phenylmethyl]glycyl-prolyl] 5a and 6a

To the solution of 1.0 mmol **4g** and 20 ml CH_2Cl_2, 2 drops conc. HCl was added with stirring. The reaction was continued for 4 h at RT. The solvent was removed *in vacuo*, then 30 mL water was added, and extracted with CH_2Cl_2 (30 ml × 3). The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated to give the product (184 mg, 53%) as a colourless solid. A $CHCl_3$ solution was left to stand for 2 days, after which, crystals **5a**/**6a** were obtained suitable for X-ray crystallographic analysis. Mp. 152~153.5 °C; IR (KBr) *v* 3526, 3346, 3151, 1715, 1672, 1608, 1576, 1503, 1103 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.66–1.70 (m, 1H), 1.88–1.91 (m, 1H), 1.97–1.99 (m, 1H), 2.30–2.33 (m, 1H), 2.56–2.61 (m, 1H), 2.65–2.70 (m, 1H), 2.95–2.97 (m, 1H), 3.13–3.15 (m, 1H), 3.26–3.31 (m, 2H), 3.37–3.41 (m, 2H), 3.53–3.58 (m, 2H), 3.78 (br, 3H), 3.78 (s, 3H), 3.79 (s, 3H), 3.82 (s, 6H), 3.85 (s, 6H), 3.90–3.97 (m, 1H), 4.22–4.25 (m, 1H), 5.99 (s, 1H), 6.43 (s, 2H), 6.57 (s, 2H) ppm; Anal. Calcd for $C_{17}H_{22}N_2O_6$: C, 58.28; H, 6.33; N, 8.00; Found: C, 58.32; H, 6.38; N, 8.06.

Note: Compound **4h**, in place of **4g** (above) as the starting material, underwent the same reaction resulting in a yield of rearrangement-fragmentation product of 48%.

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