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The development and preparation of the 2,4-dimethoxybenzyl arylhydrazine (DMBAH) "latent" safety-catch linker: solid phase synthesis of ketopiperazines

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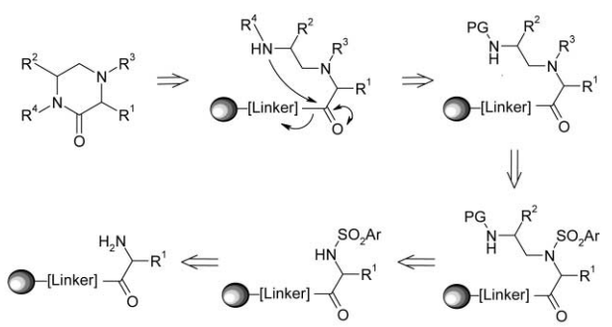
The development and preparation of the 2,4-dimethoxybenzyl arylhydrazine (DMBAH) linker **3**, a new class of "latent" safety-catch linker which is stable under Mitsunobu alkylation conditions and in the presence of amines and hydrazine, is reported. The utility of the new linker is exemplified by the synthesis of ketopiperazines (MKPs) **24** bearing up to four points of diversity using a cyclitive cleavage approach.

Introduction

Over the last thirty years, solid phase organic synthesis has evolved into a valuable chemical technology for the generation of compound libraries.[1] This has been facilitated by the availability of an increasing number of linkers which have permitted the transposition of many synthetic transformations to the solid phase.[2]

Mono-ketopiperazines (MKPs) represent an attractive small scaffold for library generation which are complementary to diketopiperazines and are able to display multiple points of diversity. An attractive synthetic route to MKP libraries is embodied in a solid phase cyclitive cleavage approach, whereby only cyclised compounds are released from the resin, leaving any acyclic impurities trapped on the support.

As outlined in Scheme 1, it was envisaged that the required resin-bound linear precursors, incorporating four points of diversity, could be prepared using Fukuyama's Mitsunobu *N*-alkylation methodology[3] to couple an immobilised α-amino acid *N*-2-nitrobenzenesulfonamide (first point of diversity) and an *N*-protected 1,2-amino alcohol (second point of diversity). In the resulting adduct replacement of the sulfonamide serves to introduce a third point of diversity. A fourth point of diversity may then be incorporated by reductive amination of the terminal amino group prior to cyclitive cleavage.



Scheme 1 Retrosynthetic analysis of mono-ketopiperazines.

Pivotal to the success of any solid phase synthesis is the choice of linker used. For the synthesis of MKPs, preliminary solution phase studies established that the Mitsunobu alkylation was most successful when performed using an amino alcohol protected with an *N*-Dde (*N*-2-acetyldimedone) group.[4] Therefore, as a prerequisite, we required a linker stable under both Mitsunobu *N*-alkylation conditions and in the presence of a strong nucleophile (hydrazinolysis of Dde protecting group), whilst being amenable to mild activation towards nucleophilic cleavage at the last step in a synthesis. A safety-catch linker, inert during the synthetic process but chemically activated prior to the cleavage step, would therefore be most suitable for our purposes.

However, a survey of existing safety-catch linkers did not reveal a candidate that would be compatible with the proposed chemistry.[5] For example, the 4-alkylsulfamylphenol safety-catch linker developed by Liener and Marshall[6] has been observed to undergo premature nucleophilic cleavage without the need for oxidative activation.[7] In addition, an acylsulfonamide linker would also be unsuitable for our purpose since their activation to nucleophilic attack is effected by *N*-alkylation.[8] Similarly, in our hands, the recently reported arylhydrazine "safety-catch" linker[9,10] was found to be incompatible with Mitsunobu *N*-alkylation conditions, giving rise to multiple products.

Herein, we report in detail the development and preparation of a new safety-catch linker which is compatible with both Mitsunobu alkylation, amines and hydrazine. The utility of the new linker was exemplified by the synthesis of MKPs bearing up to 4 points of diversity.[11] In particular, we disclose a detailed study of the cleavage of the new linker under nucleophilic cyclitive cleavage conditions in the presence of copper cations as an oxidant.

Results and discussion

Preliminary investigations showed that the incompatibility of the reported arylhydrazine linker[9,10] with Mitsunobu reaction conditions was attributable to the presence of the reactive hydrazine moiety which was susceptible to *N*-alkylation. It occurred to us that temporary protection of the anilino nitrogen would circumvent this possibility. Prior to cleavage, a suitable protecting group could be removed to reveal the hydrazine moiety which could then be subjected to oxidative cleavage (Scheme 2). In this sense, the proposed new linker would serve as a "latent" safety-catch linker.

Synthesis of the 2,4-dimethoxybenzyl arylhydrazine (DMBAH) linker

The choice of a suitable linker protecting group was pivotal to the successful implementation of our strategy. We chose to use the acid-labile 2,4-dimethoxybenzyl group (DMB) to block the reactive nitrogen, since its removal could be effected under

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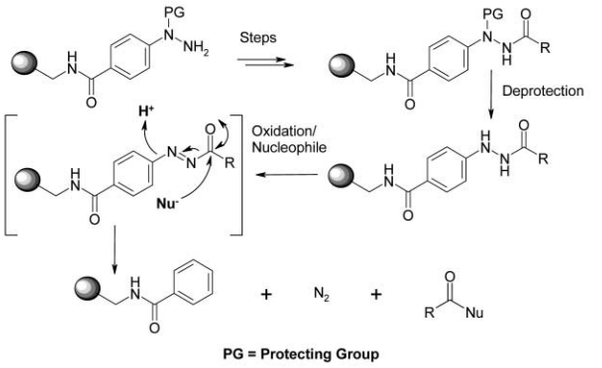
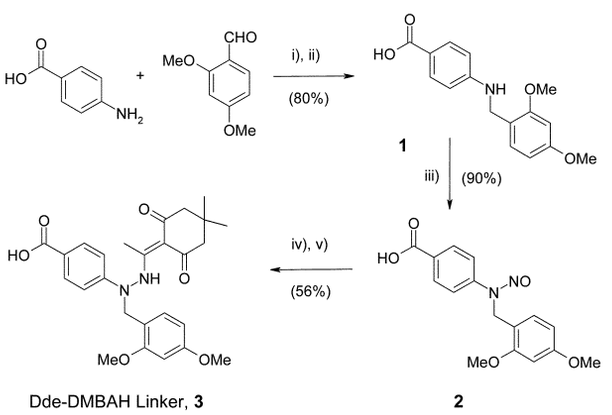


Scheme 2 Principle of the "latent" arylhydrazine safety-catch linker.

acidic conditions which are orthogonal to those required for the synthesis of MKPs.

Thus, 4-aminobenzoic acid and 2,4-dimethoxybenzaldehyde were condensed in refluxing toluene under Dean–Stark conditions to form the intermediate imine, which was immediately reduced to the corresponding aniline **1** with sodium triacetoxyborohydride. Nitrosation of **1** afforded the nitrosamine **2**, which in turn was carefully reduced to the hydrazine in the presence of zinc.[12] The linker was most conveniently isolated in multigram quantities as the Dde derivative **3** (Dde–DMBAH linker, Scheme 3).

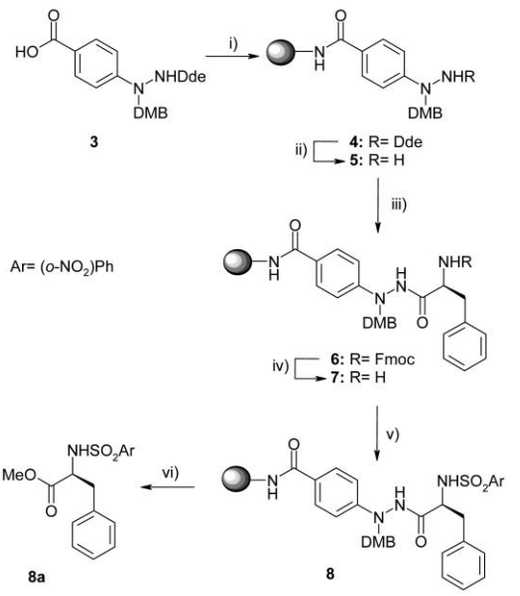


Scheme 3 *Reagents and conditions:* i) *p*-TsOH, toluene, reflux; ii) $NaBH(OAc)_3$, CH_2Cl_2, rt; iii) $NaNO_2$, H_2SO_4, EtOH, rt; iv) Zn, AcOH, aq. NaOH, 0 °C to 100 °C; v) 2-acetyldimedone, EtOH, reflux.

Solid phase synthesis of MKPs

In order to establish that the new DMBAH linker **3** was indeed suitable for the solid phase synthesis of MKPs and compatible with both Mitsunobu *N*-alkylation and Dde hydrazinolysis, we targeted a series of representative MKPs incorporating up to four points of diversity. The key starting resin **8** was prepared as shown in Scheme 4. The protected DMBAH linker **3** was first loaded onto ArgoGel™–NH_2 to afford resin **4** which was in turn treated with hydrazine hydrate to afford the free hydrazino-resin **5**. Resin **5** was treated with *N*-Fmoc-phenylalanine in the presence of diisopropylcarbodiimide (DIC) to afford the acylated resin **6**. Subsequent treatment with piperidine in a mixture of DMF and dichloromethane (1 : 1) effected complete removal of the Fmoc group to yield the amino-resin **7**, which was then sulfonylated with 2-nitrobenzenesulfonyl chloride in the presence of diisopropylethylamine (DIPEA).

At this stage, a sample of the resulting resin **8** was cleaved for analysis to confirm that the linker was compatible with the preceding chemistries. The cleavage of the DMBAH linker was performed in two steps: acidic treatment with a solution of TFA in dichloromethane (10% v/v) to remove the DMB *N*-protecting group, followed by oxidation of the resulting acyl arylhydrazine in the presence of copper(II) acetate in methanol as an external



Scheme 4 *Reagents and conditions*: i) ArgoGel™–NH_2, PyBOP, HOBt, DIPEA, CH_2Cl_2, DMF, rt; ii) 20% (v/v) hydrazine hydrate–DMF, rt; iii) Fmoc–Phe–OH, DIC, CH_2Cl_2, DMF, rt; iv) piperidine–CH_2Cl_2–DMF (1 : 2 : 2), rt; v) 2-nitrobenzenesulfonyl chloride, DIPEA, CH_2Cl_2, rt; vi) a) 10% (v/v) TFA–CH_2Cl_2, rt, b) $Cu(OAc)_2$, pyridine, MeOH, rt.

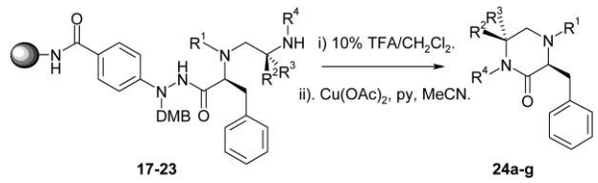
nucleophile. Gratifyingly, in a small scale experiment, the desired methyl ester **8a**[13] was obtained in greater than 95% purity by LC-MS analysis and subsequent re-exposure of the recovered resin to the two stage cleavage protocol gave no additional **8a**.

In progressing the intermediate resin **8**, we were concerned that the final ketopiperazine formation might be dependant upon the relative configuration of the stereocentres present. Therefore, we elected to work in two representative diastereomeric series. Thus, the sulfonamide resin **8** was alkylated under Mitsunobu conditions with *N*-Dde-D-phenylalaninol **9** and *N*-Dde-L-phenylalaninol **10** to yield resins **11** and **12** respectively (Scheme 5).

In order to introduce another point of diversity onto the dipeptoid template, the 2-nitrobenzenesulfonamide *N*-protecting group was removed from the resins **11** and **12** in the presence of sodium thiophenolate to afford the secondary amino-resins **13** and **14**. These, in turn, were acylated with 2-naphthoyl chloride to yield the corresponding resins **15** and **16**. Resins **11**–**16** were *N*-deprotected in the presence of hydrazine to yield the primary amino-resins **17**, **18**, **19**, **20**, **21** and **22**. Notably, no evidence for the premature cyclitive cleavage of these amino-resins was observed prior to removal of the DMB *N*-protecting group and oxidative activation of the DMBAH linker. To exemplify reductive amination,[14] the primary amine resin **18** was treated with *p*-tolylaldehyde in the presence of TMOF and $NaBH(OAc)_3$ to give the corresponding secondary amine resin **23**.

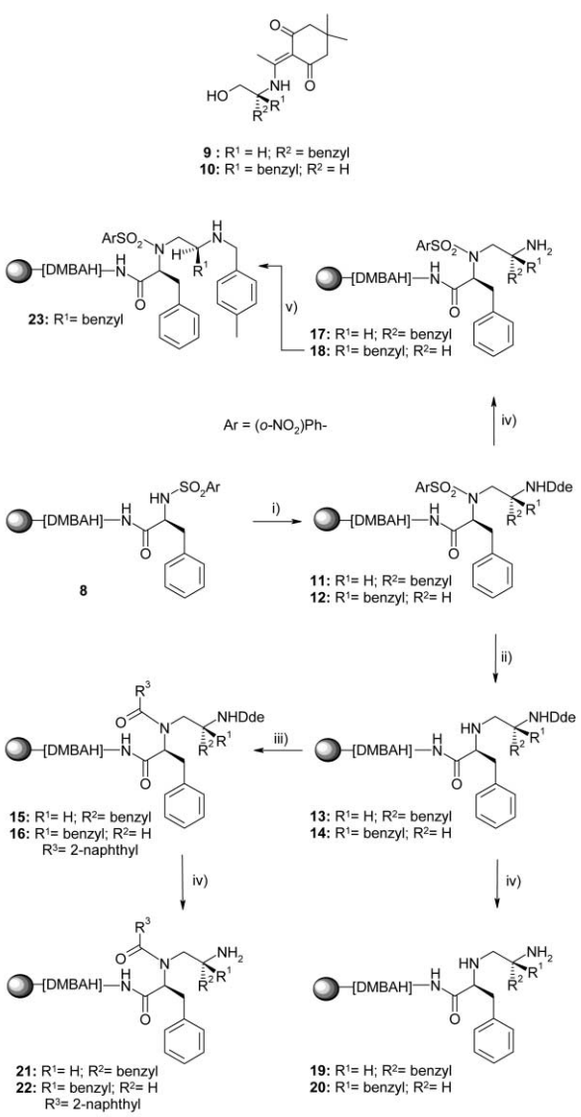
To promote the cyclitive cleavage of the resin-bound precursors **17**–**23**, the amino-resins **17**–**23** were first treated with a solution of TFA in dichloromethane (10% v/v) for 10 min, and then a solution of copper(II) acetate (2 equiv.) and pyridine (10 equiv.) in acetonitrile (a non-nucleophilic solvent in which the copper salt is reasonably soluble) for 2–3 h. In all cases, LC-MS analysis of the resulting solutions showed the presence of the desired products and the crude materials were readily purified by preparative HPLC to give the MKPs **24a**–**g** (Table 1). Notably, the relative configuration did not significantly influence the yield and purity of the products obtained (**24c** and **24d**). However, we noted that the crude purities and overall yields obtained for **24c**, **24d** and **24g** were only moderate to good—in contrast to the other examples. In particular, the

Table 1 Isolated yields and crude purities of MKPs **24a**–**g**



Resin	MKP	R^1	R^2	R^3	R^4	Purity (%)[a]	Yield (%)[b]
17	**24a**	*o*-NO_2PhSO_2	H	Benzyl	H	>99	68
18	**24b**	*o*-NO_2PhSO_2	Benzyl	H	H	90	65
19	**24c**	H	H	Benzyl	H	74	49
20	**24d**	H	Benzyl	H	H	63	54
21	**24e**	2-Naphthyl	H	Benzyl	H	96	69
22	**24f**	2-Naphthyl	Benzyl	H	H	>99	70
23	**24g**	*o*-NO_2PhSO_2	Benzyl	H	*p*-$MePhCH_2$	75	34

[a] HPLC product peak area in crude cleavage mixture (230 nm). [b] Overall purified isolated yield for the whole synthetic sequence starting from ArgoGel™–NH_2 resin.



Scheme 5 *Reagents and conditions*: i) **9** or **10**, di-*tert*-butyl azodicarboxylate (TBAD), Ph_3P, CH_2Cl_2, rt; ii) PhSNa in DMF, rt; iii) 2-naphthoyl chloride, DIPEA, CH_2Cl_2, rt; iv) hydrazine hydrate, DMF, rt; v) *p*-tolylaldehyde, CH_2Cl_2–trimethylorthoformate (3 : 1) followed by $NaBH(OAc)_3$, AcOH (1%) in CH_2Cl_2.

amine-containing MKPs **24c** and **24d** were contaminated with a number of unidentified impurities not observed for the other examples and the isolated yield of **24g** derived by cyclisation of a secondary amine was low. Thus, in order to gain a better understanding of the cyclitive cleavage process we initiated a more thorough study with the objective of identifying robust and general conditions that could be applied to the parallel synthesis of compound arrays.

A study of the cyclitive cleavage process

To simplify product clean-up,[15] we wanted to limit the amount of copper salts used for the oxidation of the linker whilst maintaining acceptable cleavage rates. We therefore studied the rate of release of the target MKPs under a range of conditions chosen so as to be compatible with any future library format.

In an initial study, we investigated the rate of cyclitive cleavage of the resins **17**, **18**, **21**–**23** when treated under identical conditions with 1.0 equivalents of copper(II) acetate and 10 equivalents of pyridine in acetonitrile (Fig. 1a). The numbers of equivalents used were calculated with respect to the maximum theoretical loading of a given resin, assuming that all the steps were quantitative and that no loss of material occurred

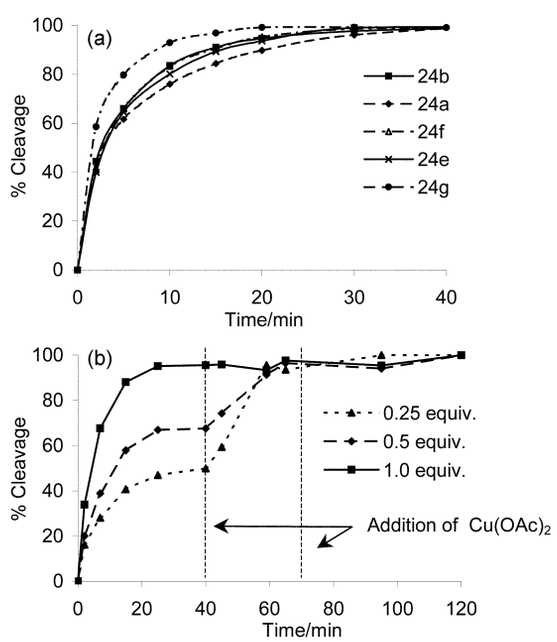


Fig. 1 (a) Cleavage profiles for resins **17**, **18**, **21**–**23** to give MKPs **24a**, **24b**, and **24e**–**g**. *Conditions*: 1 equiv. $Cu(OAc)_2$; 10 equiv. pyridine. (b) Cleavage profile for resin **18** to give MKP **24b** in the presence of 0.25, 0.5 and 1.0 equiv. of $Cu(OAc)_2$, 10 equiv. pyridine. Additional $Cu(OAc)_2$ added at $t = 40$ min and $t = 70$ min.

throughout the solid phase synthesis. The rate of cleavage of each MKP was monitored over time by HPLC analysis of the crude supernatants. Interestingly, under identical experimental conditions, the cyclitive cleavage rates of the five resins **17**, **18**, **21**–**23** to afford MKPs **24a**, **24b**, **24e**, **24f** and **24g** were found to be similar and complete cleavage of each compound was observed in less than 40 min. The similarities in cyclitive cleavage rates observed suggest a low structural dependence on the resin-bound precursors. Moreover, it suggests that the oxidation of the linker to the acyldiazene dictates the rate of release, and that subsequent intramolecular nucleophilic attack on the carbonyl is fast. This is also true for the cyclitive cleavage of the secondary amino-resin **24g**. Therefore, the low yield obtained in this case (34%) might be better attributed to a reduced resin loading arising from premature cleavage, or degradation of the linker during the prolonged reductive amination step (72 h).

In principle, the oxidation of the hydrazine to the acyldiazene is catalytic in copper(II), as the catalyst can be reoxidised by the presence of oxygen dissolved in the reaction solvent. Indeed, typically 0.5 equivalents of a copper(II) salt were sufficient to cleave the previously reported arylhydrazine linker in less than 2 h.[9] Therefore, in a second investigation (Fig. 1b), it was decided to carry out a series of kinetic experiments to study the effect of copper stoichiometry and concentration on the cyclisation–release process. Since the cyclisation profiles of compounds **24a**, **24b**, **24e**, **24f** and **24g** appeared to be very similar, resin **18** was selected as a representative example for this study.

Resin **18** was cleaved in three parallel experiments in the presence of 0.25 (expt. 1), 0.5 (expt. 2) and 1.0 (expt. 3) theoretical equivalents of copper(II) acetate in the same volume of acetonitrile–pyridine. The experiments were carried out in open vessels under vigorous stirring. The curves obtained for the release of MKP **24b** clearly indicate that the rate of release is strongly dependent on the stoichiometry and concentration of copper(II) acetate.

After 40 min, additional copper(II) acetate was added to each experiment as required to bring the total amount present in each to 1.0 equivalent. This resulted in a rapid increase in the rate of cleavage in expts. 1 and 2. After a further 30 min, another equivalent of copper(II) acetate was added to each experiment, but the amount of **24b** in the supernatants remained constant, indicating that all the substrate had been released from the resin. Extrapolation of the curves obtained from expts. 1 and 2 suggested that even a considerable increase in reaction time would still give rise to a reduced cleavage yield if less than 1.0 theoretical equivalent of copper(II) acetate was used. By contrast, in expt. 3, with 1.0 theoretical equivalent of copper, at a concentration of 1.0 mg cm^{-3}, complete release of the desired product occurred in less than 1 h.

However, in a third study (Fig. 2a), the cleavage of resins **19** and **20** in the presence of 1.0 equivalent of Cu(II) to give MKPs **24c** and **24d** respectively showed a different behaviour. In the course of the experiment, the supernatant gradually lost the blue colour characteristic of copper(II) ions. Addition of a further 1.0 equivalent of copper(II) acetate after 100 min restored the colour and an increase in conversion was immediately observed. The decolouration of the solution and dark colouration of the resin beads indicated that reduction to Cu(I) occurred, but that in this case subsequent aerobic oxidation to Cu(II) did not occur. Notably, MKPs **24c** and **24d** differ from the other examples prepared in that they contain a basic secondary amine.

A further experiment was carried out to study the stability of the MKP **24d** in the presence of excess copper cations (Fig. 2b). Thus, after removal of the DMB *N*-protecting group, resin **20** was exposed to 2.0 theoretical equivalents of copper(II) acetate in acetonitrile–pyridine. The formation of MKP **24d** was closely monitored and it was observed that a maximum concentration of the desired product was attained after only 10 min,

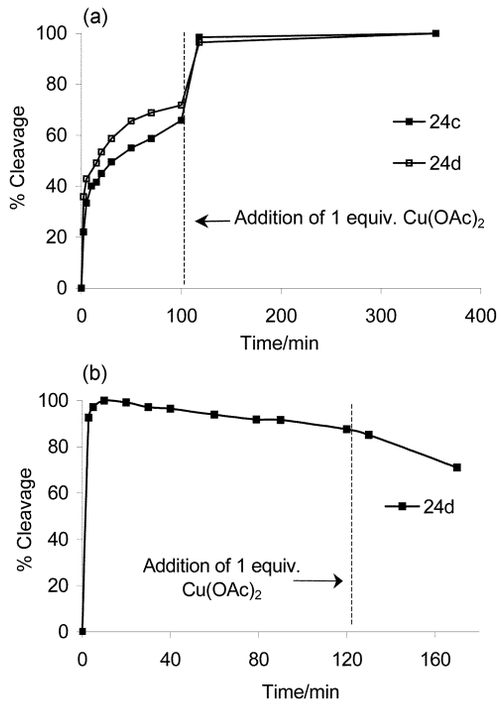


Fig. 2 (a) Cleavage profile for resins **19** and **20** to give MKPs **24c** and **24d** in the presence of 1 equiv. $Cu(OAc)_2$. Additional 1 equiv. $Cu(OAc)_2$ added at $t = 100$ min. (b) Cleavage profile for resin **20** to give MKP **24d** in the presence of 2 equiv. $Cu(OAc)_2$. Additional 1 equiv. of $Cu(OAc)_2$ added at $t = 120$ min.

but that this progressively decreased with time. After 120 min, another equivalent of copper(II) acetate was added to the cleavage mixture and a further increase in the rate of disappearance of **24d** was noted along with the appearance of a number of impurities.[16] This experiment indicates that the behaviour of MKPs containing a free secondary amine differs under the cleavage conditions from that of the corresponding acylated or sulfonylated analogues (which were found to be stable for several hours in the presence of excess Cu(II) cations – data not shown). Although an increased amount of copper ions is required for complete cleavage, shorter reaction times are necessary to avoid subsequent degradation of the product. This observation would be particularly relevant to the preparation of compounds containing unprotected amino groups using the DMBAH linker.

Conclusions

In summary, we have described the preparation and development of a new "latent" arylhydrazine solid phase linker (DMBAH, **3**) and have shown that it is stable under Mitsunobu conditions and in the presence of a good nucleophile such as hydrazine. The utility of the new linker has been demonstrated in a versatile solid phase synthesis of mono-ketopiperazines (MKPs) **24** utilising a cyclitive cleavage strategy. Up to four points of diversity were incorporated into the MKP scaffold in this way. A study to identify robust cyclitive cleavage conditions for the preparation of MKPs has determined that the treatment of the DMBAH resin with a solution of TFA in dichloromethane (10% v/v) followed by exposure to 1.0 theoretical equivalent of copper(II) acetate in acetonitrile containing 10 equivalents of pyridine reliably gives complete cleavage in under 1 h – except where the product MKP contains a secondary amine in the ring. In this case, 2.0 equivalents of copper(II) acetate and a shorter reaction time (20 min) is preferred to avoid subsequent degradation of the resulting MKP. Further studies to demonstrate the utility of the new linker **3** for the preparation of libraries based upon related heterocyclic

scaffolds by a cyclitive cleavage strategy using the protocols described herein are currently in progress and will be the subject of a future report.

Experimental

Unless otherwise specified, all reactions involving resins were carried out using Alltech™ tubes mounted on a IKA Vibrax VXR laboratory shaker. All moisture sensitive reactions were carried out under a nitrogen atmosphere in oven-dried glassware. All solvents and reagents were used as supplied unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on Polygram™ SIL G/UV_{254} plates. Column chromatography was performed over Merck Kieselgel 9385 under nitrogen pressure. Analytical high pressure liquid chromatography (HPLC) was performed using a Hewlett Packard Series 1050 instrument. Column: Supercosil™ ABZ^+PLUS 3.3 cm × 4.6 mm, 3 μm. Eluent A: water, 0.1% TFA; B: acetonitrile 95%, water 5%, TFA 0.05%. Flow rate: 1 cm^3 min^{-1}. Detection: UV (diode array: 215, 230, 254 nm). Method: gradient 10–95% B in A over 7 min. Auto-preparative HPLC purifications were carried out on a Gilson AutoPrep system. Column: Supercosil™ ABZ^+PLUS 10 cm × 2.12 cm, 5 μm. Eluent: A, B. Flow rate: 6 cm^3 min^{-1}. Detection: 215 nm. Method: (20%–95% B in A) over 20 min. Melting points were measured on a Mettler FP5 automatic melting point apparatus in open capillaries and are uncorrected. Optical rotations were measured at the sodium D-line using an Optical Activity AA10 polarimeter at 20 °C (*c* is measured in g 100 cm^{-3}, in the indicated solvent, impling units of deg dm^2 g^{-1}). Infrared spectra were collected on a Bio Rad FT-IR machine using DRIFTs from a potassium bromide (KBr) surface. Liquid chromatography-mass spectra (LC-MS) were recorded on a Micromass Platform I (8084) under electrospray positive and negative ionisation conditions. Column: Supercosil™ ABZ^+PLUS 3.3 cm × 4.6 mm, 3 μm. Eluent A: 10 mM solution of ammonium acetate in water, 0.1% formic acid; B: acetonitrile 95%, water 5%, formic acid 0.05%. Flow rate: 1 cm^3 min^{-1}. Detection: UV (diode array: 215, 230, 254 nm). Method: gradient 0–100% B in A over 3.5 min. Accurate mass spectra were recorded on a VG Autospec mass spectrometer in positive electrospray mode. NMR spectra were recorded in the indicated solvent. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane, the following abbreviations are used for multiplicities: s = singlet; d = doublet; t = triplet; m = multiplet; dd = doublet of doublets; br = broad; and coupling constant *J*-values are quoted in Hz. ^{1}H NMR spectra were recorded on either Bruker AM 400 or Varian Inova 750 spectrometers at 400 MHz and 750 MHz respectively. Variable temperature ^{1}H NMR were recorded at the specified temperature using a Bruker DPX 250 at 250 MHz. ^{1}H HRMAS NMR were recorded on a Bruker AM 400 fitted with a Varian Nano NMR probe and a CPMG pulse sequence was utilised to minimise line broadening and aid interpretation. ^{13}C NMR were recorded on a Bruker AM 400 at 100 MHz with proton decoupling.

Where possible, to characterise resins analytical cleavage of small samples of beads was performed according to the following procedure and the supernatant solution obtained analysed by both HPLC and LC-MS:

Analytical cleavage of resin-bound substrates for HPLC–LC-MS analysis

A small sample of resin (typically between fifty and a hundred beads) was transferred to a RAININ RT-10GF pipette filter tip. The beads were washed with DMF (1 cm^3) and CH_2Cl_2 (1 cm^3), and then treated with a solution of TFA in CH_2Cl_2 (10% v/v; 1 cm^3). After 10 min, the beads were filtered, washed (DMF: 1 × 1 cm^3, CH_2Cl_2: 1 × 1 cm^3), and treated with a saturated solution of $Cu(OAc)_2$ in pyridine–methanol (1 : 30; 50 μl). The mixture was left to stand for 30 min and then filtered. The filtrate was collected in an HPLC vial and submitted for HPLC and LC-MS analysis. *Nb*. Under these cleavage conditions, the methyl ester of the substrate cleaved from the resin was obtained.

4-(2,4-Dimethoxybenzylamino)benzoic acid (1). *p*-TsOH (200 mg) was added to a suspension of 4-aminobenzoic acid (12.4 g, 90.2 mmol) and 2,4-dimethoxybenzaldehyde (15.0 g, 90.2 mmol) in toluene (500 cm^3) and the resulting mixture was heated under reflux with Dean–Stark water removal for 3 h. The solvent was evaporated under reduced pressure and the residual solid was suspended in CH_2Cl_2 (500 cm^3) and treated with acetic acid (15 cm^3) and sodium triacetoxyborohydride (28.0 g, 135 mmol). The resulting suspension was stirred at room temperature for 72 h when the solvent was removed under reduced pressure. The yellow oil obtained was triturated with water (500 cm^3) for 1 h and the resulting off-white powder was collected on a filter. The powder was washed with water (2 × 20 cm^3) and diethyl ether (2 × 20 cm^3) and then triturated with diethyl ether to give the benzoic acid **1** as a white solid (20.7 g, 80%). Mp: 185–187 °C (from EtOH); HPLC (254 nm): t_R = 4.45 min (100%) [Found: C, 67.06; H, 5.81; N, 4.74%; $(M + H)^+$ 288.1244. $C_{16}H_{17}NO_4$ requires C, 66.89; H, 5.96; N, 4.87%; *MH*, 288.1235]; ν_{max} (KBr)/cm^{-1} 3403, 1672, 1604, 1315, 1295 and 1176; δ_H (400 MHz, DMSO-d_6) 11.92 (1 H, s), 7.61 (2 H, d, *J* 9), 7.08 (1 H, d, *J* 8), 6.71 (1 H, t, *J* 6), 6.55–6.50 (3 H, m), 6.46 (1 H, dd, *J* 9), 4.15 (2 H, d, *J* 6), 3.78 (3 H, s) and 3.71 (3 H, s); δ_C (100 MHz, DMSO-d_6) 167.6, 159.9, 158.1, 152.7, 131.2, 128.9, 118.8, 117.1, 111.1, 103.4, 98.5, 55.6, 55.3 and 40.7; *m*/*z* (ESI) 288 $[M + H]^+$.

4-(2,4-Dimethoxybenzylnitrosoamino)benzoic acid (2). To an ice-cold suspension of **1** (19.6 g, 68.2 mmol) in absolute ethanol (500 cm^3) was added sodium nitrite (4.94 g, 71.6 mmol) followed, slowly, by conc. H_2SO_4 (4.65 cm^3, 87.3 mmol). The resulting mixture was stirred at room temperature for 18 h, when additional $NaNO_2$ (2.40 g, 34.1 mmol) and conc. H_2SO_4 (2.3 cm^3 as a solution in 20 cm^3 of ethanol) were added. After 2 h, ice-water (400 cm^3) was added and the mixture stirred for 10 min before being concentrated under reduced pressure to a volume of approximately 500 cm^3. Water (500 cm^3) was added and the resulting suspension was collected by filtration to give the acid **2** as a brown powder (19.55 g, 90%). Mp: 165–167 °C (from EtOH); HPLC (254 nm): t_R = 4.76 min (100%) [Found: C, 60.50; H, 4.92; N, 8.36. $C_{16}H_{16}N_2O_5$ requires C, 60.76; H, 5.10; N, 8.86%]; ν_{max} (KBr)/cm^{-1} 1680, 1606, 1294 and 901; δ_H (400 MHz, DMSO-d_6) 12.92 (1 H, s), 7.99 (2 H, d, *J* 9), 7.67 (2 H, d, *J* 9), 6.73 (1 H, d, *J* 9), 6.49 (1 H, d, *J* 3), 6.36 (1 H, dd, *J* 9, 3), 5.14 (2 H, s), 3.69 (3 H, s) and 3.67 (3 H, s); δ_C (100 MHz, DMSO-d_6) 189.6, 168.9, 162.5, 160.0, 146.8, 132.9, 131.2, 121.7, 115.9, 107.1, 100.8, 57.8, 57.5 and 43.8; *m*/*z* (ESI) 339 $[(M + Na)^+$, 80%], 287 $[(M - NO + H)^+$, 100%].

4-{*N*-(2,4-Dimethoxybenzyl)-*N*′-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]hydrazino}benzoic acid (3). To a solution of **2** (3.80 g, 12.0 mmol) in aqueous sodium hydroxide (1 M, 12 cm^3) and water (100 cm^3) was added zinc (16 g, 0.24 mol, freshly activated with dilute HCl). The resulting suspension was cooled to 0 °C and acetic acid (6 cm^3) was added dropwise over 20 min with vigorous stirring, causing the suspension to thicken to a paste. The mixture was heated slowly to 100 °C (bath temperature) and maintained at this temperature for 6 hours. The suspension was then filtered and the resulting grey solid triturated with aqueous NaOH (2 M, 2 × 10 cm^3) which caused a white solid to precipitate. The pH was adjusted to pH 4 using concentrated HCl and the suspension was filtered to yield a white solid, which was dried under reduced pressure. The solid was suspended in ethanol (60 cm^3) and treated with 2-acetyl-

dimedone (Dde–OH: 1.13 g, 6.20 mmol) under reflux periodically adding additional Dde–OH until a slight excess persisted according to TLC analysis. The solvents were removed under reduced pressure to leave an oil which was purified by column chromatography (EtOAc–hexane–acetic acid: 31 : 66 : 3) to give the Dde-protected linker **3** as a white solid (3.12 g, 56%). Mp: 199–201 °C (from MeCN–water); HPLC (254 nm): t_R = 4.81 min (100%) [Found: C, 67.10; H, 6.41; N, 5.71%; $(M + H)^+$ 467.2173. $C_{26}H_{30}N_2O_6$ requires C, 66.94; H, 6.48; N, 6.00%; *MH*, 467.2182]; ν_{max} (KBr)/cm^{-1} 1709, 1684, 1604, 1588 and 1288; δ_H (400 MHz, DMSO-d_6) 14.00 (1 H, s), 12.45 (1 H, s), 7.81 (2 H, d, *J* 9), 7.11 (1 H, d, *J* 8), 6.89 (2 H, d, *J* 9), 6.49 (1 H, d, *J* 2), 6.41 (1 H, dd, *J* 8, 2), 4.67 (2 H, br s), 3.73 (3 H, s), 3.69 (3 H, s), 2.30 (4 H, br s), 2.22 (3 H, s) and 0.93 (6 H, s); δ_C (100 MHz, DMSO-d_6) 175.1, 166.9, 160.7, 158.5, 151.2, 131.9, 130.9, 128.8, 128.1, 121.9, 114.4, 112.7, 106.1, 104.3, 98.1, 55.2, 55.1, 52.1, 29.6, 27.7 and 15.9; *m*/*z* (ESI) 467 [$(M + H)^+$, 100%].

4-{*N*-(2,4-Dimethoxybenzyl)-*N*′-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]hydrazino}benzoic acid, amide with ArgoGel™–NH_2 (4). To a slurry of ArgoGel™–NH_2 (1.50 g, theoretical (th.) loading 0.41 mmol g^{-1}) in CH_2Cl_2 (10 cm^3) was added diisopropylethylamine (1 cm^3) and the resulting mixture was shaken for 5 min. The resin was thoroughly washed with distilled CH_2Cl_2 (3 × 10 cm^3) and then treated with a solution of **3** (0.57 g, 1.20 mmol), PyBOP (0.96 g, 1.80 mmol) and HOBt (0.24 g, 1.80 mmol) in DMF–CH_2Cl_2 (1 : 1; 10 cm^3). After 4 min, diisopropylethylamine (0.64 cm^3, 3.60 mmol) was added and the suspension was stirred at room temperature for 18 h. The resin was washed (DMF: 6 × 5 cm^3, CH_2Cl_2: 6 × 10 cm^3) and dried under reduced pressure to give the resin **4** (1.8 g); Kaiser test: −ve;[17] δ_H (400 MHz, $CDCl_3$, MAS) 7.70 (d), 6.92 (d), 6.81 (d), 6.32 (s), 6.26 (d), 5.20 (s), 3.75–3.70 (2 × s), 2.29 (br s), 2.25 (s) and 0.95 (s).

4-[*N*-(2,4-Dimethoxybenzyl)hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (5). To a slurry of resin **4** (1.20 g, max. th. loading 0.31 mmol g^{-1}) in DMF (8 cm^3) was added hydrazine hydrate (55% N_2H_4 in water, 2 cm^3). After 15 min the resin was washed with DMF (3 × 5 cm^3). This procedure was repeated three times and the resin was finally filtered, washed (DMF: 6 × 5 cm^3, CH_2Cl_2: 6 × 5 cm^3) and dried under reduced pressure to give the resin **5** (1.13 g). δ_H (400 MHz, $CDCl_3$, MAS) 7.70 (d), 7.05 (m), 6.48 (s), 6.40 (d), 4.65 (s) and 3.75–3.70 (2 × s).

4-{*N*-(2,4-Dimethoxybenzyl)-*N*′-[(2,*S*)-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropionyl]hydrazino}benzoic acid, amide with ArgoGel™–NH_2 (6). To a slurry of resin **5** (1.13 g, max. th. loading: 0.36 mmol g^{-1}) in CH_2Cl_2–DMF (1 : 1, 2 cm^3) was added a solution of Fmoc–Phe–OH (1.61 g, 4.16 mmol) and DIC (0.65 cm^3, 4.16 mmol) in CH_2Cl_2–DMF (1 : 1, 10 cm^3). The suspension was shaken for 18 h and then washed (DMF: 6 × 5 cm^3, CH_2Cl_2: 6 × 5 cm^3) and dried under reduced pressure to give the resin **6** (1.28 g). Fmoc UV quantification [18] indicated a loading of 0.26 mmol g^{-1}; Kaiser test: −ve.

4-[*N*′-((2,*S*)-Amino-3-phenylpropionyl)-*N*-(2,4-dimethoxybenzyl)hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (7). To a slurry of resin **6** (1.18 g, max. th. loading: 0.32 mmol g^{-1}) in CH_2Cl_2 (1 cm^3) was added a solution of piperidine in CH_2Cl_2–DMF (1 : 2 : 2; 10 cm^3). The resulting suspension was stirred for 10 min, before the resin was washed (DMF: 3 × 5 cm^3). The above procedure was repeated three additional times. Finally, the resin was thoroughly washed (DMF: 6 × 5 cm^3, CH_2Cl_2: 6 × 5 cm^3) and dried under reduced pressure to give the resin **7** (1.19 g); Kaiser test: +ve.

4-{*N*-(2,4-Dimethoxybenzyl)-*N*′-[(2,*S*)-(2-nitrobenzenesulfonylamino)-3-phenylpropionyl]hydrazino}benzoic acid, amide with ArgoGel™–NH_2 (8). To a suspension of resin **7** (1.19 g, max. th. loading 0.35 mmol g^{-1}) in CH_2Cl_2 (2 cm^3) was added diisopropylethylamine (0.36 cm^3, 2.08 mmol) followed by a solution of *o*-nitrobenzenesulfonyl chloride (0.46 g, 2.08 mmol) and diisopropylethylamine (0.36 cm^3, 2.08 mmol) in CH_2Cl_2 (10 cm^3). After 1 h the suspension was filtered, washed (DMF: 6 × 5 cm^3, CH_2Cl_2: 6 × 5 cm^3) and dried under reduced pressure to give the resin **8** (1.28 g). Analytical cleavage of a sample of **8** gave the following data: HPLC (254 nm): t_R = 4.96 min (100%); LC-MS: t_R = 4.98 min, $[M + H]^+$ = 365, $[M + NH_4]^+$ = 382 (data consistent with that expected for the corresponding methyl ester **8a**: *M* 364).

2-[1-((1,*R*)-Hydroxymethyl-2-phenylethylamino)ethylidene]-5,5-dimethylcyclohexane-1,3-dione (9). To a solution of (*R*)-2-amino-3-phenylpropanol (1.0 g, 6.6 mmol) in absolute ethanol (5 cm^3) was added, dropwise, a solution of 2-acetyldimedone (1.2 g, 6.6 mmol) in absolute ethanol (5 cm^3). The mixture was stirred at room temperature for 24 h before the solvent was evaporated under reduced pressure to give a yellow oil. The oil was taken up in a minimum of EtOAc and hexane was added until the solution became turbid. The solvents were removed under reduced pressure and the resulting solid triturated with hexane to give the alcohol **9** as a white powder (1.64 g, 79%). R_f 0.43 (EtOAc); HPLC (230 nm): t_R = 3.95 min (100%); $[a]_D$ +299 (*c* 1.00 in MeOH) [Found: C, 72.29; H, 8.07; N, 4.26%; $(M + H)^+$ 316.1909. $C_{19}H_{25}NO_3$ requires C, 72.35; H, 7.99; N, 4.44%; *MH*, 316.1912]; ν_{max} (KBr)/cm^{-1} 3362, 2955, 2878, 1631, 1573, 1465 and 1337; δ_H (400 MHz, $CDCl_3$) 13.62 (1 H, br s), 7.28–7.14 (5 H, m), 4.05 (1 H, m), 3.77 (1 H, dd, *J* 4, 11.5), 3.71 (1 H, dd, *J* 5.5, 11.5), 3.17 (1 H, br s), 3.00 (1 H, dd, *J* 6, 14), 2.80 (1 H, dd, *J* 9, 14), 2.32 (4 H, s), 1.26 (3 H, s) and 1.00 (6 H, s); δ_C (100 MHz, $CDCl_3$) 198.4, 173.9, 137.2, 129.6, 129.1, 127.4, 108.3, 64.3, 57.7, 57.6, 53.1, 38.8, 30.4, 28.6 and 18.1; *m*/*z* (ESI) 316 [$(M + H)^+$, 100%].

2-[1-((1,*S*)-Hydroxymethyl-2-phenylethylamino)ethylidene]-5,5-dimethylcyclohexane-1,3-dione (10). To a solution of (*S*)-2-amino-3-phenylpropanol (1.00 g, 6.61 mmol) in ethanol (10 cm^3) was added 2-acetyldimedone (1.26 g, 6.94 mmol). After 2 h, additional (*S*)-2-amino-3-phenylpropanol (0.20 g, 1.32 mmol) was added to the mixture. After a further 1 h, the yellow solution was passed through an SCX solid phase extraction cartridge (strongly acidic sulfonic acid) to scavenge excess amine. The cartridge was washed with methanol (*ca.* 20 cm^3) and the combined filtrates were concentrated under reduced pressure to leave a thick yellow oil. The oil was taken up in CH_2Cl_2 (20 cm^3) and evaporated to dryness. This process was repeated 3× to remove any residual ethanol. The resulting clear glass was then triturated in boiling hexane to give the alcohol **10** as a white solid (1.90 g, 91%). R_f 0.43 (EtOAc); HPLC (230 nm): t_R = 3.95 min (100%); $[a]_D$ −299 (*c.* 1.00 in MeOH) [Found: C, 72.29; H, 7.99; N, 4.34%; $(M + H)^+$ 316.1906. $C_{19}H_{25}NO_3$ requires C, 72.35; H, 7.99; N, 4.44%; *MH*, 316.1912]; ν_{max} (KBr)/cm^{-1} 3364, 2955, 2878, 1631, 1573, 1465 and 1337; δ_H (400 MHz, $CDCl_3$) 13.62 (1 H, br s), 7.28–7.14 (5 H, m), 4.05 (1 H, m), 3.77 (1 H, dd, *J* 4, 11.5), 3.71 (1 H, dd, *J* 5.5, 11.5), 3.17 (1 H, br s), 3.00 (1 H, dd, *J* 6, 14), 2.80 (1 H, dd, *J* 9, 14), 2.32 (4 H, s), 1.26 (3 H, s) and 1.00 (6 H, s); δ_C (100 MHz, $CDCl_3$) 198.4, 173.9, 137.2, 129.6, 129.1, 127.4, 108.3, 64.3, 57.7, 57.6, 53.1, 38.8, 30.4, 28.6 and 18.1; *m*/*z* (ESI) 316 [$(M + H)^+$, 100%].

General procedure for on-resin Mitsunobu alkylation

4-(*N*-(2,4-Dimethoxybenzyl)-*N*′-{(2,*S*)-[{(2,*R*)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]-3-phenylpropyl}-(2-nitrobenzenesulfonyl)amino]-3-phenylpropionyl}hydrazino)-benzoic acid, amide with ArgoGel™–NH_2 (11). To a slurry of resin **8** (640 mg, max. th. loading: 0.33 mmol g^{-1}) in CH_2Cl_2 (3 cm^3), was added triphenylphosphine (569 mg, 2.17 mmol)

and *N*-Dde-D-phenylalaninol **9** (710 mg, 2.17 mmol) as a solution in CH_2Cl_2 (3 cm^3). A solution of di-*tert*-butyl azodicarboxylate (500 mg, 2.17 mmol) in CH_2Cl_2 (1.2 cm^3) was added in five portions and the resulting yellow suspension was stirred for 19 h. The resin was filtered, washed (DMF: 6 × 5 cm^3, CH_2Cl_2: 6 × 5 cm^3) and dried under reduced pressure to give the resin **11** (780 mg). Analytical cleavage of a sample of **11** gave the following data: HPLC (254 nm): t_R = 6.17 min (100%); LC-MS: t_R = 5.52 min, $[M + H]^+$ = 662 (data consistent with that expected for the corresponding methyl ester: *M* 661).

4-(*N*-(2,4-Dimethoxybenzyl)-*N*′-{(2,*S*)-[{(2,*S*)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]-3-phenylpropyl}-(2-nitrobenzenesulfonyl)amino]-3-phenylpropionyl}hydrazino)-benzoic acid, amide with ArgoGel™–NH_2 (12). According to the general procedure for resin **11**, resin **8** (640 mg, max. th. loading: 0.33 mmol g^{-1}) was treated with triphenylphosphine (569 mg, 2.17 mmol), *N*-Dde-phenylalaninol **10** (710 mg, 2.17 mmol) and di-*tert*-butyl azodicarboxylate (500 mg, 2.17 mmol) in CH_2Cl_2 for 19 h to give the resin **12** (766 mg). Analytical cleavage of a sample of **12** gave the following data: HPLC (254 nm): t_R = 6.11 min (100%); LC-MS: t_R = 5.45, min, $[M + H]^+$ = 662 (data consistent with that expected for the corresponding methyl ester: *M* 661).

General procedure for the on-resin removal of the *o*-nitrobenzenesulfonyl group

4-[*N*-(2,4-Dimethoxybenzyl)-*N*′-((2,*S*)-{(2,*R*)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]-3-phenylpropyl-amino}-3-phenylpropionyl)hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (13). To a slurry of resin **11** (200 mg, max. th. loading 0.30 mmol g^{-1}) in DMF (2 cm^3) was added a solution of sodium thiophenolate (158 mg, 1.2 mmol, 20 equiv.) in DMF (2 cm^3). The resin was stirred for 15 min and then the dark blue solution was filtered. The resin was thoroughly washed (DMF 3 × 5 cm^3, allowed to stand for 10 min, 3× [MeOH: 3 cm^3, 2 minutes then CH_2Cl_2: 3 cm^3, 2 minutes]) and dried under reduced pressure to give the resin **13** (188 mg). Analytical cleavage of a sample of **13** gave the following data: HPLC (254 nm): t_R = 4.14 min (100%); LC-MS: t_R = 5.17 min, $[M + H]^+$ = 477 (data consistent with that expected for the corresponding methyl ester: *M* 476).

4-[*N*-(2,4-Dimethoxybenzyl)-*N*′-((2,*S*)-{(2,*S*)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]-3-phenylpropyl-amino}-3-phenylpropionyl)hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (14). According to the general procedure for resin **13**, the resin **12** (200 mg, max. th. loading 0.30 mmol g^{-1}) was treated with sodium thiophenolate (158 mg, 1.2 mmol, 20 equiv.) to give the resin **14** (190 mg). Analytical cleavage of a sample of **14** gave the following data: HPLC (254 nm): t_R = 4.10 min (100%); LC-MS: t_R = 5.17 min, $[M + H]^+$ = 477 (data consistent with that expected for the corresponding methyl ester: *M* 476).

General procedure for on-resin acylation with acid chlorides

4-(*N*-(2,4-Dimethoxybenzyl)-*N*′-{(2,*S*)-[{(2,*R*)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]-3-phenylpropyl}-(naphthalene-2-carbonyl)amino]-3-phenylpropionyl}hydrazino)-benzoic acid, amide with ArgoGel™–NH_2 (15). To a slurry of resin **13** (189 mg) in CH_2Cl_2 (2 cm^3) was added a solution of 2-naphthoyl chloride (228 mg, 1.2 mmol) in CH_2Cl_2 (2 cm^3). The resulting suspension was stirred for 5 h and then the resin was filtered, washed (DMF: 6 × 2 cm^3, CH_2Cl_2: 6 × 2 cm^3) and dried to give the resin **15** (200 mg). Analytical cleavage of a sample of **15** gave the following data: HPLC (254 nm): t_R = 6.40 min (100%); LC-MS: t_R = 5.64 min, $[M + H]^+$ = 631 (data consistent with that expected for the corresponding methyl ester: *M* 630).

4-(*N*-(2,4-Dimethoxybenzyl)-*N*′-{(2,*S*)-[{(2,*S*)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino]-3-phenylpropyl}-(naphthalene-2-carbonyl)amino]-3-phenylpropionyl}hydrazino)-benzoic acid, amide with ArgoGel™–NH_2 (16). According to the general procedure for resin **15**, the resin **14** (188 mg) was treated with 2-naphthoyl chloride (228 mg, 1.2 mmol) to give the resin **16** (201 mg). Analytical cleavage of a sample of **16** gave the following data: HPLC (254 nm): t_R = 6.40 min (100%); LC-MS: t_R = 5.63 min $[M + H]^+$ = 631 (data consistent with that expected for the corresponding methyl ester: *M* 630).

General procedure for the on-resin removal of the Dde *N*-protecting group

4-[*N*′-{(2,*S*)-[((2,*R*)-Amino-3-phenylpropyl)-(2-nitrobenzenesulfonyl)amino]-3-phenylpropionyl}-*N*-(2,4-dimethoxybenzyl)-hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (17). To a slurry of resin **11** (200 mg, max. th. loading 0.30 mmol g^{-1}) in DMF (1.6 cm^3) was added hydrazine hydrate (55% N_2H_4 in water, 0.4 cm^3) and the resulting mixture stirred for 15 min before the resin was washed with DMF (3 × 2 cm^3). The procedure was repeated. Finally, the resin was filtered, washed (DMF: 6 × 2 cm^3, CH_2Cl_2: 6 × 2 cm^3) and dried under reduced pressure to give the resin **17** (189 mg). Analytical cleavage of a sample of **17** gave the following data: HPLC (230 nm): t_R = 5.39 min (88%); LC-MS: t_R = 5.09 min, $[M + H]^+$ = 466 (data consistent with that expected for the MKP **24a**: *M* 465).

4-[*N*′-{(2,*S*)-[((2,*S*)-Amino-3-phenyl-propyl)-(2-nitrobenzenesulfonyl)-amino]-3-phenyl-propionyl}-*N*-(2,4-dimethoxybenzyl)-hydrazino]benzoic acid, amide with ArgoGel™-NH_2 (18). According to the general procedure for resin **17**, the resin **12** (200 mg, max. th. loading 0.30 mmol g^{-1}) was treated with hydrazine hydrate (55% N_2H_4 in water, 0.4 cm^3) to give the resin **18** (190 mg). Analytical cleavage of a sample of **18** gave the following data: HPLC (230 nm): t_R = 5.42 min (89%); LC-MS: t_R = 5.12 min, $[M + H]^+$ = 466 (data consistent with that expected for the MKP **24b**: *M* 465).

4-[*N*′-[(2,*S*)-((2,*R*)-Amino-3-phenylpropylamino)-3-phenyl-propionyl]-*N*-(2,4-dimethoxybenzyl)hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (19). According to the general procedure for resin **17**, the resin **13** (100 mg, max. th. loading 0.31 mmol g^{-1}) was treated with hydrazine hydrate (55% N_2H_4 in water, 0.4 cm^3) to give the resin **19** (95 mg). Analytical cleavage of a sample of **19** gave the following data: HPLC (254 nm): t_R = 2.81 min (63%); LC-MS: t_R = 4.02 min, $[M + H]^+$ = 281 (data consistent with that expected for the MKP **24c**: *M* 280).

4-[*N*′-[(2,*S*)-((2,*S*)-Amino-3-phenylpropylamino)-3-phenyl-propionyl]-*N*-(2,4-dimethoxybenzyl)hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (20). According to the general procedure for resin **17**, the resin **14** (100 mg, max. th. loading 0.31 mmol g^{-1}) was treated with hydrazine hydrate (55% N_2H_4 in water, 0.4 cm^3) to give the resin **20** (95 mg). Analytical cleavage of a sample of **20** gave the following data: HPLC (254 nm): t_R = 2.46 min (74%); LC-MS: t_R = 3.82 min, $[M + H]^+$ = 281 (data consistent with that expected for the MKP **24d**: *M* 280).

4-[*N*′-{(2,*S*)-[((2,*R*)-Amino-3-phenylpropyl)(naphthalene-2-carbonyl)amino]-3-phenylpropionyl}-*N*-(2,4-dimethoxybenzyl)-hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (21). According to the general procedure for resin **17**, the resin **15** (200 mg, max. th. loading 0.30 mmol g^{-1}) was treated with hydrazine hydrate (55% N_2H_4 in water, 0.4 cm^3) to give the resin **21** (191 mg). Analytical cleavage of a sample of **21** gave the following data: HPLC (254 nm): t_R = 5.51 min (100%); LC-MS: t_R = 5.79 min, $[M + H]^+$ = 435 (data consistent with that expected for the MKP **24e**: *M* 434).

4-[*N*′-{(2,*S*)-[((2,*S*)-Amino-3-phenylpropyl)(naphthalene-2-carbonyl)amino]-3-phenylpropionyl}-*N*-(2,4-dimethoxybenzyl)-hydrazino]benzoic acid, amide with ArgoGel™–NH_2 (22). According to the general procedure for resin **17**, the resin **16** (200 mg, max. th. loading 0.30 mmol g^{-1}) was treated with hydrazine hydrate (55% N_2H_4 in water, 0.4 cm^3) to give the title resin **22** (185 mg). Analytical cleavage of a sample of **22** gave the following data: HPLC (254 nm): t_R = 5.40 min (92%); LC-MS: t_R = 5.16 min, $[M + H]^+$ = 435 (data consistent with that expected for the MKP **24f**: *M* 434).

4-(*N*-(2,4-Dimethoxybenzyl)-*N*′-{2-[[2-(4-methylbenzylamino)-3-phenylpropyl]-(2-nitrobenzenesulfonyl)amino]-3-phenylpropionyl}hydrazino)benzoic acid, amide with ArgoGel™–NH_2 (23). To a slurry of resin **18** (140 mg, max. th. loading: 0.31 mmol g^{-1}) in CH_2Cl_2–trimethylorthoformate (3 : 1; 2 cm^3) was added *p*-tolylaldehyde (50 µl, 0.42 mmol). The resulting suspension was shaken for 3.5 h then filtered and washed with anhydrous CH_2Cl_2 (6 × 2 cm^3). The resin was then immediately suspended in CH_2Cl_2 (3 cm^3) and treated with sodium triacetoxyborohydride (89 mg, 0.42 mmol) and acetic acid (30 µl). The resulting slurry was stirred for 72 h at room temperature. Finally, the resin was filtered and washed (DMF: 3 × 1 cm^3, H_2O: 3 × 1 cm^3, DMF: 3 × 1 cm^3, CH_2Cl_2: 6 × 2 cm^3) to give the resin **23**. Analytical cleavage of a sample of **23** gave the following data: HPLC (215 nm): t_R = 6.61 min (85%); LC-MS: t_R = 5.64 min, $[M + H]^+$ = 570 (data consistent with that expected for MKP **24g**: *M* 569).

General procedure for the cyclitive cleavage of resin-bound intermediates (unoptimised)

(3*S*,*R*)-3,6-Dibenzyl-4-(2-nitrobenzenesulfonyl)piperazin-2-one (24a). The resin **17** (190 mg, max. th. loading 0.31 mmol g^{-1}) was treated with a solution of TFA in CH_2Cl_2 (10% v/v; 2 cm^3) for 10 min and then washed thoroughly (DMF: 6 × 1 cm^3; CH_2Cl_2: 6 × 1 cm^3). The resin was then treated with a solution of copper(II) acetate (10.8 mg, 0.06 mmol) and pyridine (48 µl, 0.60 mmol) in acetonitrile (10 cm^3). After stirring for 2 h at room temperature, the resin was filtered, washed (CH_2Cl_2: 3 × 2 cm^3) and the resulting combined organic phases evaporated to dryness under reduced pressure to give a thick blue oil. The oil was purified by autopreparative HPLC and the combined fractions lyophilised to give the MKP **24a** as a white powder (19 mg, 68%). HPLC (230 nm): t_R = 5.41 min (100%); $[\alpha]_D$ +295 (*c.* 0.41 in $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3360, 3209, 3090, 1675, 1543, 1368, 1165, 751 and 701; δ_H (400 MHz, $CDCl_3$) 7.90 (1 H, d, *J* 7.5), 7.66 (2 H, m), 7.57 (1 H, m), 7.32–7.23 (3 H, m), 7.05 (7 H, m), 6.57 (1 H, br s), 4.82 (1 H, ddd, *J* 6.5, 5, 1), 3.77 (1 H, dd, *J* 15, 3), 3.34 (2 H, m), 3.22 (1 H, dd, *J* 6.5, 4), 3.12 (1 H, dd, *J* 5, 4), 2.76 (1 H, dd, *J* 9.5, 7) and 2.67 (1 H, dd, *J* 9.5, 14); δ_C (100 MHz, $CDCl_3$) 167.5, 145.7, 134.1, 134.0, 132.1, 131.7, 130.4, 129.6, 127.9, 127.4, 127.2, 126.6, 125.4, 122.9, 117.7, 57.9, 51.3, 41.8, 39.0 and 36.3; *m*/*z* (ESI) [Found: $(M + H)^+$, 466.1449. $C_{24}H_{24}N_3O_5S$ requires *MH*, 466.1436], (ESI) 466 ($[M + H]^+$, 100%).

(3*S*,6*S*)-3,6-Dibenzyl-4-(2-nitrobenzenesulfonyl)piperazin-2-one (24b). According to the general procedure for **24a**, the resin **18** (185 mg, max. th. loading 0.31 mmol g^{-1}) was treated with a solution of TFA in CH_2Cl_2 (10% v/v), then copper(II) acetate (10.8 mg, 0.06 mmol) and pyridine (48 µl, 0.60 mmol) to give, after purification by HPLC and lyophilisation of the combined fractions, the MKP **24b** as a white powder (18 mg, 65%). HPLC (230 nm): t_R = 5.36 min (100%); $[\alpha]_D$ +148 (*c* 0.33 in $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3373, 3202, 3030, 1673, 1545, 2368, 1165 and 755; δ_H (400 MHz, $CDCl_3$) 7.66–7.58 (3 H, m), 7.47 (1 H, m), 7.35–7.25 (3 H, m), 7.07 (7 H, m), 6.14 (1 H, br s), 4.65 (1 H, m), 4.02 (1 H, dd, *J* 15, 4.5), 3.74 (1 H, m), 3.19 (1 H, dd, *J* 4.5, 14), 3.13 (1 H, dd, *J* 8, 14), 2.79 (2 H, m) and 2.36 (1 H, dd, *J* 9, 13.5); δ_C (100 MHz, $CDCl_3$) 169.4, 147.9, 136.6, 134.8, 134.0, 133.8, 132.6, 131.4, 130.0, 129.6, 129.5, 128.8, 128.0, 127.6, 124.9, 60.3, 53.8, 44.9, 39.9 and 38.4; *m*/*z* (ESI) [Found: $(M + H)^+$, 466.1436. $C_{24}H_{24}N_3O_5S$ requires *MH*, 466.1436], *m*/*z* (ESI) 466 ($[M + H]^+$, 100%).

(3*S*,6*R*)-3,6-Dibenzylpiperazin-2-one (24c). According to the general procedure for **24a**, the resin **19** (94 mg, max. th. loading 0.33 mmol g^{-1}) was treated with a solution of TFA in CH_2Cl_2 (10% v/v), then copper(II) acetate (10.8 mg, 0.06 mmol) and pyridine (24 µl, 0.30 mmol) in acetonitrile (5 cm^3) for 3 h to give, after purification by HPLC and lyophilisation of the combined fractions, the trifluoroacetate salt of the MKP **24c** as a white powder (18 mg, 65%). HPLC (230 nm): t_R = 2.47 min (100%); $[\alpha]_D$ −68 (*c.* 0.31 in MeOH); ν_{max} (KBr)/cm^{-1} 3205, 2941, 1677 and 1202; δ_H (750 MHz, DMSO-d_6) 9.19 (1 H, br), 8.43 (1 H, br), 7.36–7.20 (10 H, m), 4.12 (1 H, br m), 3.82 (1 H, br m), 3.40 (1 H, dd, *J* 15, 4), 3.05 (1 H, br), 3.01 (1 H, dd, *J* 14, 4), 2.93 (1 H, dd, *J* 15, 9), 2.84 (1 H, br), and 2.71 (1 H, dd, *J* 14, 8.5); δ_C (100 MHz, $CDCl_3$) 165.8, 134.5, 134.1, 129.9, 129.7, 129.4, 129.3, 128.4, 128.2, 70.0, 50.7, 45.8, 40.1 and 35.8; *m*/*z* (ESI) [Found: $(M + H)^+$, 281.1658. $C_{18}H_{21}N_2O$ requires *MH*, 281.1653], (ESI) 281 ($[M + H]^+$, 100%).

(3*S*,6*S*)-3,6-Dibenzylpiperazin-2-one (24d). According to the general procedure for **24a**, the resin **20** (95 mg, max. th. loading 0.33 mmol g^{-1}) was treated with a solution of TFA in CH_2Cl_2 (10% v/v), then copper(II) acetate (10.8 mg, 0.06 mmol) and pyridine (24 µl, 0.30 mmol) in acetonitrile (5 cm^3) for 3 h to give, after purification by HPLC and lyophilisation of the combined fractions, the trifluoroacetate salt of the MKP **24d** as a white powder (6.4 mg, 54%). HPLC (230 nm): t_R = 2.83 min (100%); $[\alpha]_D$ −7.5 (*c.* 4 in MeOH); ν_{max} (KBr)/cm^{-1} 3376, 2924, 1677, 1202, 1136 and 1032; δ_H (750 MHz, DMSO-d_6) 9.22 (1 H, br), 8.43 (1 H, br), 7.35–7.20 (10 H, m), 4.12 (1 H, br m), 3.82 (1 H, br m), 3.21 (1 H, dd, *J* 15, 5.5), 3.06 (1 H, br d, *J* 13.5), 3.03 (1 H, dd, *J* 15, 8), 2.92 (1 H, dd, *J* 13.5, 4.5), 2.79 (1 H, dd, *J* 13.5, 7.5) and 2.73 (1 H, dd, *J* 13.5, 9); δ_C (100 MHz, $CDCl_3$) 164.5, 133.1, 132.8, 128.3, 127.9, 127.8, 127.7, 126.6, 126.4, 68.3, 49.0, 41.2, 38.6, and 34.2; *m*/*z* (ESI) [Found: $(M + H)^+$, 281.1662. $C_{18}H_{21}N_2O$ requires *MH*, 281.1653], (ESI) 281 ($[M + H]^+$, 100%).

(3*S*,6*R*)-3,6-Dibenzyl-4-(naphthalene-2-carbonyl)piperazin-2-one (24e). According to the general procedure for **24a**, the resin **21** (95 mg, max. th. loading 0.33 mmol g^{-1}) was treated with a solution of TFA in CH_2Cl_2 (10% v/v), then copper(II) acetate (10.8 mg, 0.06 mmol) and pyridine (48 µl, 0.60 mmol) in acetonitrile (10 cm^3) for 3 h to give, after purification by HPLC and lyophilisation of the combined fractions, the MKP **24e** as a yellow powder (17 mg, 69%). HPLC (230 nm): t_R = 5.37 min (100%); $[\alpha]_D$ +143 (*c.* 0.30 in $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3242, 3062, 3029, 2932, 1673, 1640, 1421, 755 and 702; the NMR spectrum at room temperature was very broad and showed the presence of several stable rotamers on the NMR time scale. At high temperature, some distinct signals could be identified although the spectrum remained badly resolved: δ_H (250 MHz, DMSO-d_6, 120 °C) 7.95–7.83 (3 H, m), 7.62–7.54 (3 H, m), 7.32–7.15 (6 H, m), 7.16–6.93 (5 H, m), 4.88 (1 H, br s), 3.85 (1 H, br d, *J* 15), 3.50 (1 H, br m), 3.27 (1 H, dd, *J* 14, 7.5), 3.21 (1 H, d, *J* 15), 3.16 (1 H, dd, *J* 14, 6.5), 2.81 (1 H, dd, *J* 13.5, 6) and 2.63 (1 H, dd, *J* 13.5, 8.5); *m*/*z* (ESI) [Found: $(M + H)^+$, 435.2077. $C_{29}H_{27}N_2O_2$ requires *MH*, 435.2072], (ESI) 435 ($[M + H]^+$, 100%).

(3*S*,6*S*)-3,6-Dibenzyl-4-(naphthalene-2-carbonyl)piperazin-2-one (24f). According to the general procedure for **24a**, the resin **22** (191 mg, max. th. loading 0.30 mmol g^{-1}) was treated with a solution of TFA in CH_2Cl_2 (10% v/v), then copper(II) acetate (10.8 mg, 0.06 mmol) and pyridine (48 µl, 0.60 mmol) in acetonitrile (10 cm^3) for 3 h to give after purification by HPLC and lyophilisation of the combined fractions, the MKP **24f** as a yellow powder (18 mg, 70%). HPLC (230 nm): t_R = 5.48 min

(100%); $[a]_D$ +91 (*c.* 0.35 in $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3221, 3062, 3029, 2931, 1671, 1426, 756 and 702; the NMR spectrum at room temperature was very broad and showed the presence of several stable rotamers on the NMR time scale. At high temperature, some distinct signals could be identified although the spectrum remained badly resolved: δ_H (250 MHz, DMSO-d_6, 120 °C) 7.92–7.73 (3 H, m), 7.60–7.45 (3 H, m), 7.31–7.23 (3 H, m), 7.20–7.03 (8 H, m), 4.83 (1 H, m), 3.81 (1 H, m), 3.72 (1 H, br d, *J* 14.5), 3.13 (2 H, m), 2.89 (1 H, dd, *J* 15, 5), 2.74 (1 H, dd, *J* 14, 10.5) and 2.54 (1 H, dd, *J* 14, 8.5); *m*/*z* (ESI) [Found: $(M + H)^+$, 435.2077. $C_{29}H_{27}N_2O_2$ requires *MH*, 435.2072], (CI) 435 ($[M + H]^+$, 100%).

(3*S*,6*S*)-3,6-Dibenzyl-1-(4-methylbenzyl)-4-(2-nitrobenzenesulfonyl)piperazin-2-one (24g). (Chelex™ work-up). According to the general procedure for **24a**, the resin **23** (151 mg, max. th. loading 0.30 mmol g^{-1}) was treated with a solution of TFA in CH_2Cl_2 (10% v/v), then copper(II) acetate (7.6 mg, 42 μmol) and pyridine (34 μl, 0.42 mmol) in acetonitrile (10 cm^3) for 70 min. Chelex™ ion exchange resin (*ca*. 1 g, prewashed) was added and then, after 3 h, the resins were filtered and washed (CH_2Cl_2: 3 × 5 cm^3, MeOH: 3 × 5 cm^3) to give after purification by HPLC, the MKP **24g** as a yellow powder (8.2 mg, 34%). HPLC (230 nm): t_R = 6.71 min (100%); $[a]_D$ +65 (*c.* 0.29 in MeOH); ν_{max} (KBr)/cm^{-1} 3292, 3029, 2922, 2853, 1649, 1545, 1454, 1370, 1167 and 737; δ_H (400 MHz, $CDCl_3$) 7.59–7.55 (2 H, m), 7.52 (1 H, m), 7.42 (1 H, m), 7.25 (3H, m), 7.14–1.05 (9H, m), 6.90–6.87 (2H, m), 5.48 (1 H, d, *J* 15), 4.63 (1 H, m), 4.11 (1 H, d, *J* 15), 3.47 (2 H, m), 3.30 (1 H, dd, *J* 13.5, 4.5), 3.15 (1 H, dd, *J* 14, 8.5), 2.96 (1 H, dd, *J* 13.5, 8), 2.77 (1 H, dd, *J* 14, 3.5), 2.32 (3 H, s) and 2.25 (1 H, dd, *J* 14, 8.5); δ_C (100 MHz, $CDCl_3$) 166.0, 147.8, 135.3, 134.3, 133.5, 131.5, 130.5, 129.8, 129.7, 129.1, 127.8, 127.4, 127.1, 126.8, 126.2, 126.0, 125.2, 124.9, 122.4, 58.9, 52.5, 44.4, 41.6, 35.5, 34.4 and 19.1; *m*/*z* (ESI) 570 ($[M + H]^+$, 100%), (ESI) [Found: $(M + H)^+$, 570.2049. $C_{32}H_{32}N_3O_5S$ requires *MH*, 570.2062].

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