Rapid Synthesis of Diketopiperazine Macroarrays *via* Ugi Four-Component Reactions on Planar Solid Supports

Qi Lin and Helen E. Blackwell*

Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison, WI 53706-1322 USA

e-mail: blackwell@chem.wisc.edu

Supplementary Information.

* To whom correspondence should be addressed.

General experimental information.

General. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-300 spectrometer in deuterated solvents at 300 MHz and 75 Hz, respectively. Chemical shifts are reported in parts per million (ppm, δ) using tetramethyl silane (TMS) as a reference (0.0 ppm). Couplings are reported in hertz. LC-MS (ESI) were obtained using a Shimadzu LCMS-2010 (Columbia, MD) equipped with two pumps (LC-10ADvp), controller (SCL-10Avp), autoinjector (SIL-10ADvp), UV diode array detector (SPD-M10Avp), and single quadrupole analyzer (by electrospray ionization, ESI). The LC-MS is interfaced with a PC running the Shimadzu LCSolutions software package (Version 2.04 Su2-H2). A Supelco (Bellefonte, PA) 15 cm × 2.1 mm C-18 wide-pore reverse-phase column was used for all LC-MS work. Standard reverse-phase HPLC conditions for LC-MS were as follows: flow rate = 200 μL/min; mobile phase A = 0.4% formic acid; mobile phase B = 0.2% formic acid in acetonitrile. HPLC analyses were performed using a Shimadzu HPLC equipped with a single pump (LC-10ATvp), solvent mixer (FCV-10ALvp), controller (SCL-10Avp), autoinjector (SIL-10AF), and UV diode array detector (SPD-M10Avp). A Shimadzu Premier 25 cm × 4.6 mm C-18 reverse-phase column was used for all HPLC work. Standard reverse-phase HPLC conditions were as follows: flow rate = 1.0 mL/min; mobile phase A = 0.1% trifluoroacetic acid (TFA); mobile phase B = 0.1% TFA in acetonitrile. UV detection at 280 nm was used for all HPLC analyses. Attenuated total reflectance (ATR)-IR spectra were recorded with a Bruker Tensor 27 spectrometer, outfitted with a single reflection MIRacle Horizontal ATR by Pike Technologies. A ZnSe crystal with spectral range 20,000 to 650 cm^{-1} was used. UV spectra were recorded using an Varian Cary 50 UV-Vis spectrometer running Cary WinUV software.

All reagents were purchased from commercial sources (Alfa-Aesar, Aldrich, Acros, NovaBiochem and Advanced ChemTech) and used without further purification. Fmoc-aminoethyl-photolinker (**2**) and Fmoc-aminoethyl-photolinker derived AM polystyrene resin were purchased from EMD Biosciences. Solvents were purchased from commercial sources (Aldrich and J.T. Baker) and used as is, with the exception of dichloromethane (CH_2Cl_2), which was distilled over calcium hydride immediately prior to use. Water was purified using a Millipore Analyzer Feed System.

Planar cellulose membranes (Whatman 1Chr chromatography paper, 20 x 20 cm squares) were purchased from Fisher Scientific and stored in a desiccator at room temperature until required for use.

Microwave instrumentation. All microwave-assisted reactions were performed in a Milestone MicroSYNTH Labstation multimodal microwave synthesis reactor equipped with a continuous power source (1000 W max).[1] This instrument is interfaced with an Ethos MicroSYNTH Lab Terminal PC running EasyWave reaction monitoring software. Using this reactor system, microwave (MW) irradiation can be applied to reactions using either power (wattage) control or temperature control. The MW reactor is equipped with a fiber-optic temperature sensor that allows direct monitoring of the internal temperature of reaction vessels and an infrared sensor (installed in the side wall of the reactor cavity) that can monitor the surface temperature of reaction vessels inside the cavity. Solvent depths of *ca.* 1 cm in the reaction vessel are required for accurate temperature monitoring using the submerged fiber-optic

temperature sensor. The reactor is equipped with a magnetic stirrer that allows for stirring during MW-assisted reactions.

All full submersion, or "blanket-type", reactions on planar cellulose supports (except for initial amination to give support **1**) were performed in the MicroSYNTH reactor using specialized 70 mL Teflon/polyetheretherketone (PEEK) reaction vessels. These vessels have appropriate holes in their lids to accommodate the fiber-optic temperature sensor in a protective ceramic sheath. This allowed for straightforward temperature control of the MW-assisted reactions. For parallel syntheses, one vessel was assembled with the temperature sensor as a reference vessel for temperature control, while the holes in the lids of the other vessels were sealed. The vessels were placed on a rotor within the reactor that rotates during MW-assisted reactions (shown in Fig. S1).

Fig. S1 View of the inside of the Milestone MicroSYNTH reactor showing the rotor and the 70 mL Teflon/PEEK reaction vessels. The yellow cord is the fiber-optic temperature sensor.

The initial amination reaction to give planar support **1** was performed in a shallow Pyrex dish on a rotating turntable inside the MicroSYNTH MW reactor using power control. Spatially addressed reactions on planar supports were irradiated using power control between two Weflon plates (20 x 20 x 0.5 cm) to increase the heating of the support. (Weflon is a polymer composite of Teflon impregnated with carbon black particles. This material is commercially available from Milestone, Inc. in a variety of shapes and sizes.[1]) Power control, as opposed to temperature control, was used during these MW-assisted reactions because the low solvent volumes on the array prohibited accurate temperature monitoring with the fiber-optic sensor. (Note, however, heating protocols were developed first using temperature control by sandwiching the fiber-optic temperature probe between the two Weflon plates (one with a groove that fit the probe), ramping to a desired temperature using MW irradiation, and then holding at that temperature for a specified amount of time. The average wattages obtained during the ramp and hold times were then used in MW-assisted SPOT reactions to reproduce these heating curves.)

For the traditional solid-phase reactions, beads and a micro stir bar were placed in a Bohdan 4-mL solid-phase synthesis vessel (#MTB2001). The vessel was placed in a 70 mL Teflon/PEEK reaction vessel with the fiber-optic temperature sensor in a protective ceramic sheath. The stirrer was set to 20% of its maximum setting, and the reaction mixture was heated in the MW reactor utilizing temperature control (see Fig. S4 below).

UV irradiation. Photocleavage from planar supports and polymeric beads was carried out with a handheld UV lamp (C. Entela Mineralight; Lamp Model UVGL-58) at long wavelength (366 nm).

Derivatization of planar cellulose support.

Representative planar cellulose membrane amination protocol. A 15 x 18 cm sheet of Whatman 1Chr paper was immersed in 60 mL of 20% TFA in CH_2Cl_2 for 5 min in a covered 2.6 L Pyrex dish (to swell the support). The solution was decanted and the sheet was dried under a stream of N_2 for 30 min and in a vacuum desiccator overnight at 25 °C. The sheet was then

immersed in 100 mL of 2.0 M tosyl chloride in pyridine for 2 h. The sheet was washed by immersion in three consecutive baths of EtOH (50 mL, 5 min each), and dried under a stream of N_2 and *in vacuo* for 30 min each. Next, the tosylated cellulose support was immersed in 60 mL of neat diamine spacer unit, 4,7,10-trioxa-1,13-tridecanediamine, in a Pyrex dish. The dish was covered with a glass sheet and placed on the rotating platform of the MicroSYNTH MW reactor. The support was subjected to MW irradiation for 15 min at 300 W, after which the amine solution was carefully decanted from the support. The support was washed in a Pyrex dish (5 min each) by adding and then decanting 50 mL portions of EtOH, 1.0 M NaOH, H_2O (2x), EtOH (2x), and MeOH (2x). The amine spacer-derived support (**1**) was dried under a stream of N_2 and *in vacuo* for 30 min each.[2] Amine loading was determined by standard Fmoc quantitation methods (see below).

Representative Fmoc coupling and quantification protocols. A single spot (6 mm diameter) of amine spacer-derived support **1** was punched-out using a desktop hole-punch and immersed in 200 μL of 0.60 M Fmoc-OSu in DMF for 2 h. The spot was washed with 2 mL portions of DMF (3x) and MeOH (3x), and then dried under a stream of N_2 for 15 min. For UV Fmoc quantification, 1.0 mL of 4% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF was added to the spot in a one-dram vial. The mixture was gently agitated for 15 min. The solution was diluted to 10x with DMF and agitated for an additional 30 sec. The solution then was transferred to a quartz cuvette and the absorbance was read at 296 nm (ε_{296}= 9880 $M^{-1}cm^{-1}$). We routinely obtained amine spacer loadings of *ca.* 8 μmol/cm^2 using the above amination protocol.

Representative procedure for loading photo-labile linker on amino cellulose support. Dots were marked on a 6 × 8 cm sheet of amino support **1** at distances 1.2 x 1.2 cm apart using a #2 lead pencil. A 0.75 M coupling solution of photolinker was prepared by dissolving 4-{4-[1-(Fmoc-amino)ethyl]-2-methoxy-5-nitrophenoxy} butanoic acid (**2**, 39.0 mg, 0.075 mmol) and HOSu (8.7 mg, 0.075 mmol) in dry NMP (100 μL), followed by the addition of *N,N'*-diisopropylcarbodiimide (DIC) (10.8 μL, 0.075 mmol) and diisopropylethylamine (DIPEA) (26.2 μL, 0.150 mmol). The solution was allowed to pre-activate at 25 °C for 15 min. Aliquots (3.0 μL) of this solution were pipetted onto *ca.* 30 individual spots on amine support (**1**). The planar support was sandwiched immediately between two Weflon plates (Fig. S2) and subjected to MW irradiation at 300 W for 5.5 min (to ramp to 95 °C), followed by 100 W for 2.5 min (to hold at 95 °C), and then washed by immersion and swirling in 30 mL portions of DMF (3x) in a Pyrex dish on a bench top orbital shaker (simultaneously covered with a cardboard box to shield the support from light). The support was subjected directly to the next step. Note, the support was covered with a cardboard box to protect the photolabile linker from light in all room temperature steps hereafter.

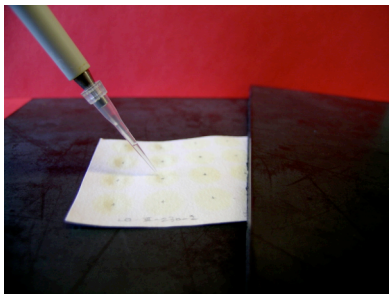


Fig. S2 Example of the manual spotting of reagents onto a planar membrane using a micropipette. The planar black surfaces are Weflon plates.

Representative acetylation and Fmoc deprotection of pro-linker support. A solution of 20% acetic anhydride and 10% DIPEA in DMF was prepared. Aliquots (3.0 μL) of this solution were pipetted onto the Fmoc-protected pro-linker support and allowed to sit at 25 °C for 20 min. This procedure was repeated, after which the support was washed by immersion and swirling in

30 mL portions of DMF (3x). A small section of the support was cut out, further washed with MeOH (3x), dried under a stream of N_2. Two spots were punched-out of this section. One spot was subject to a Kaiser colorimetric test.[3] (In this test, a yellow colored spot indicated complete capping of residual amines.) The other spot was subjected to UV Fmoc quantification. The remaining support was subjected to "blanket-type" Fmoc deprotection by swirling in 20% piperidine in DMF for 10 min (2x). The support then was washed in 30 mL portions of DMF (3x) and MeOH (3x) and dried under a stream of N_2 and *in vacuo* for 30 min each to yield amine linker-derived support **3**.

Representative coupling of *N*-α-Fmoc-4-nitro-L-phenylalanine (4) onto support 3. A 1.0 M coupling solution was prepared by dissolving *N*-α-Fmoc-4-nitro-L-phenylalanine (Fmoc-L-Nph-OH) (**4**, 34.6 mg, 0.080 mmol) and HOSu (9.2 mg, 0.080 mmol) in dry NMP (80 μL), followed by the addition of DIC (12.4 μL, 0.080 mmol), and DIPEA (27.8 μL, 0.160 mmol). The solution was allowed to pre-activate at 25 °C for 15 min. Aliquots (3.0 μL) of the solution were pipetted onto the *ca*. 30 spots of support **3**. The support was sandwiched immediately between two Weflon plates and subjected to MW irradiation at 300 W for 6 min (to ramp to 100 °C), followed by 100 W for 4 min (to hold at 100 °C). The support then was washed by immersion and swirling in 30 mL portions of DMF (3x). A spot was removed for Fmoc quantification and the remaining support was subjected to acetylation and Fmoc deprotection as outlined above to generate amine-derived support **5**.

Synthesis of model DKP product (10a) on planar cellulose support.

Representative Ugi 4CR on planar support 5. Neat cyclohexane carboxaldehyde and a 2.0 M solution of Fmoc-Gly-OH in DMF were mixed in a 1:4 volume ratio (~1:1 mole ratio) to make 25 μL (Solution A). A 4.0 μL aliquot of Millipore water was spotted onto amine-derived support **5**, followed by 3.0 μL of Solution A and 1.2 μL of 1-isocyanocyclohexene/hexanes mixture (2:1 mole ratio by ^{1}H NMR).[4] The spotted support was allowed to sit at 25 °C for 10 min. This procedure was then repeated twice with reduced amounts of each reagent (*i.e.*, 2.0 μL water, 1.2 μL Solution A and 0.8 μL isocyanide). Reducing the volume of reagents applied to the support during re-spotting prevented contamination between spots, while good conversion to Ugi product was maintained. The support then was washed by immersion and swirling in DMF, MeOH, DMF (2x) and MeOH (2x). Support **6** was dried overnight in a vacuum oven at 100 °C.

Fig. S3 Example of reaction format used for "blanket-type" MW reactions on planar supports. A planar cellulose array can been seen in the Teflon sheath (white) that will be placed inside the PEEK vessel (brown).

Representative methanolysis reaction on planar support 6. Support **6** was rolled into a tube and immersed in 22 mL of 10% AcCl solution in MeOH in 70 mL Teflon/PEEK MW reaction vessels (Fig. S3). The reactions were heated in the MW reactor to reach 80 °C in 5 min. This temperature was held using MW irradiation for 10–30 min. The solution was carefully decanted, and the support was washed with MeOH (3x) and CH_2Cl_2 (1x) while inside the vessel. This methyl ester-derived support (**7**) was dried under a stream of N_2 inside the vessel for

30 min and then removed.

Representative DKP formation on planar support 7. Methyl ester support **7** was placed in a Teflon watch glass and spotted with 3.0 μL of 20% piperidine in DMF and allowed to sit at 25 °C for 20 min. This procedure was repeated twice, followed by washing with DMF (3x) and MeOH (3x). The DKP array **9** was dried and acetylated using the method described above (to generate support **3**).

Photocleavage protocol for DKP products. A spot of DKP array **9** was punched-out and placed in a glass vial containing 0.5 mL of MeOH. The vial was placed on an upturned, handheld UV lamp that was set on an oscillating shaker plate and irradiated at 366 nm for 16 h. The MeOH solution was collected and concentrated *in vacuo*. The resulting residue (**10**) was dissolved in 200 μL of CH_3CN/H_2O (1:1, v/v), filtered, and analyzed by HPLC and LC-MS.

Optimization of model DKP syntheses.

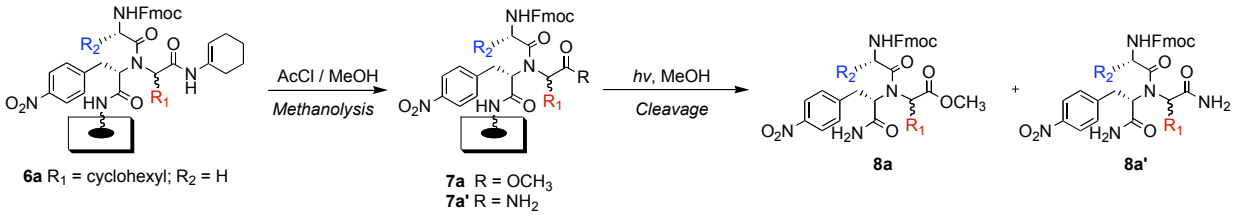
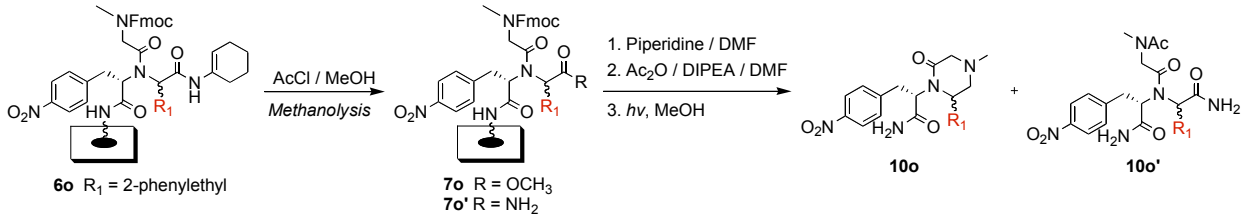


Table S1 Optimization of methanolysis reaction leading to compound **8a**

Conditions	Reaction Time		Ratio 8a : 8a' [a, b]
RT	24 h		24 :76
Heating Block			
50 °C	1 h		43 : 57
50 °C	2 h		65 : 35
50 °C	3 h		77 : 23
50 °C	5 h		91 : 9
70 °C	23 min		75 : 25
80 °C	14 min		86 : 14
80 °C	38 min		100 : 0
Microwave	**Ramp Time**	**Hold Time**	
50 °C	2 min	10 min	14 : 86
60 °C	2.5 min	10 min	39 : 61
70 °C	3 min	10 min	79 : 21
70 °C	3 min	20 min	85 : 15
75 °C	4 min	10 min	81 : 19
80 °C	4 min	10 min	100 : 0
90 °C	4.5 min	10 min	100 : 0
100 °C	5 min	10 min	100 : 0

[a] Based on integration of HPLC spectra with UV detection at 280 nm after cleavage; Error = ±2%. [b] Ratio of **8a** takes into account *ca.* 5-15% DKP product **10a** formed due to thermal cleavage of the *N*-Fmoc group (during oven drying prior to methanolysis) and subsequent cyclization after methanolysis.



6o R_1 = 2-phenylethyl

7o R = OCH_3
7o' R = NH_2

10o

10o'

Table S2 Optimization of methanolysis reaction leading to DKP **10o** (Bg)

Conditions	**Reaction Time**		**Puirty 10o** (%)[a]	**Purity 10o'** (%)[a]
RT	24 h		30	70
Heating Block 80 °C	35 min		70	30
Microwave	**Ramp Time**	**Hold Time**		
80 °C	4 min	10 min	50	50
80 °C	5 min	15 min	67	33
80 °C	5 min	30 min	85	15
85 °C	5 min	10 min	67	33
90 °C	5 min	10 min	74	26
90 °C	5 min	20 min	91	9
100 °C	5 min	10 min	90	10

[a] Based on integration of HPLC spectra with UV detection at 280 nm after cleavage; Error = ±2%.

Synthesis of model DKP product (10a) on photolinker-derived AM resin.

In an aluminum foil-wrapped Micro Bio-Spin Column (Bio-Rad, #732-6204), 201.6 mg (151.2 μmol) of Fmoc-aminoethyl-photolinker AM resin (0.75 mmol/g) was swelled in 2.4 mL of dry DMF for 20 min, followed by the addition of 0.6 mL of piperidine (to make a 20% solution). The supernatant was filtered after 20 min of agitation at 25 °C. The resin was washed with 2 mL of DMF, and then treated with 3.0 mL of 20% piperidine in DMF again for 20 min, followed by washing with 2 mL portions of DMF (3x) and MeOH (3x). The amino-linker resin was dried *in vacuo* for 1 h.

Five equivalents of Fmoc-L-Nph-OH (**4**, 328.1 mg, 756.0 μmol) and the coupling reagent *O*-benzotriazol-1-yl-*N,N,N'N'*-tetramethyluronium hexafluorophosphate (HBTU) (287.3 mg, 756.0 μmol) were dissolved in 1 mL of dry DMF, to which DIPEA (263.2 μL, 1.51 mmol) in dry DMF (756 μL) was added. The dark yellow mixture was allowed to sit at 25 °C for 5 min, after which it was added to the Micro Bio-Spin Column containing the amino-linker resin. The resin slurry was agitated for 4 h. The reagent solution was drained from the resin, and the beads were washed with 2 mL portions of DMF (3x), CH_2Cl_2 (3x), and MeOH (3x), followed by drying *in vacuo* for 30 min. The resin tested negative for free amines using a Kaiser colorimetric test.[3] The Fmoc group was cleaved with 20% piperidine in DMF and the resin was washed using the same method described in the previous paragraph.

The Nph-derived resin was placed in an aluminum foil-wrapped, fritted extract-clean reservoir (Alltech, #210208), to which a solution containing 2.67 mL water, 133 μL cyclohexane carboxaldehyde, and 533 μL of 0.5 M *N*-Fmoc-Gly-OH solution in DMF was added. Next, a 333 μL aliquot of a 1-isocyanocyclohexene/hexanes solution (2:1 mole ratio by ^{1}H NMR) was added. The vessel was agitated for 45 min at 25 °C. The resin was washed with 2 mL portions of DMF

(3x), MeOH (1x), CH_2Cl_2 (1x), MeOH (2x), and CH_2Cl_2 (2x), and dried overnight in a vacuum oven at 45 °C.

The resin was divided into two portions. Each portion was added into a Bohdan 4-mL solid-phase synthesis vessel (#MTB2001) with a micro stir bar. The vessels were then submerged in 25 mL of 10% AcCl in MeOH in 70 mL Teflon/PEEK MW reaction vessels (see Fig. S4). The stirrer was set to 20% of its maximum setting and the reactions were heated to 80 °C in 4 min and held at this temperature for 10 min in the MW reactor. Temperature was monitored using the fiber-optic sensor that was submersed in the solution of one of these vessels. The vessels were then cooled in ice water for 5 min. This heating and cooling procedure was repeated twice. The resin was washed with 2 mL portions of MeOH (2x) and DMF (3x), and subjected to 2 mL of 20% piperidine in DMF at 25 °C for 30 min (2x), followed by washing with 2 mL portions of DMF (3x). Next, the resin was treated with 20% Ac_2O and 10% DIPEA in DMF at 25 °C for 20 min (2x). The resulting DKP resin was washed with 2 mL portions of DMF (3x), MeOH (1x), CH_2Cl_2 (1x), MeOH (2x), and CH_2Cl_2 (2x), and dried *in vacuo* for 30 min.

Fig. S4 Example of reaction format used for MW-assisted reactions performed on beads. The beads are placed in a glass synthesis vessel and then placed inside a Teflon/PEEK MW reaction vessel.

Compound cleavage. The resin was placed in glass vials (20 mL) containing 3 mL of MeOH. The vials were placed on an upturned, handheld UV lamp that was set on an oscillating shaker plate and irradiated at 366 nm for 48 h. The MeOH solution was collected and concentrated *in vacuo*. An HPLC trace of the cleaved crude product is shown in Fig. S5. The crude product was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH, 80:1 to 20:1, v/v) to yield the DKP product **10a** as a white solid (3.8 mg). Overall yield: 7%.

TLC: R_f=0.25 (CH_2Cl_2/MeOH, 9:1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): (major diastereomer) δ 8.16 (d, 2, J = 9.0), 7.42 (d, 2, J = 9.0), 6.78 (br, 1), 6.46 (br, 1), 5.55 (br, 1), 5.19 (t, 1, J_1 = 8.1), 4.14 (d, 1, J = 18), 3.95 (t, 2, J = 4.8), 3.50 (dd, 1, J_1 = 8.1, J_2 = 14.4), 3.18 (dd, 1, J_1 = 8.1, J_2 = 14.4), 1.8-1.0 (m, 11); ^{13}C NMR (300 MHz, $CDCl_3$): δ 171, 167, 166, 147, 144, 130, 124, 63.5, 57.6, 45.7, 42.0, 33.1, 30.5, 27.7, 26.5, 26.2, 25.9; IR (neat): 2925, 2855, 1667, 1606, 1519, 1450, 1346, 1333, 1201, 1128, 737, 700; MS (ESI): *m/z* $(M+H)^+$ calculated 389.2, found 389.1.

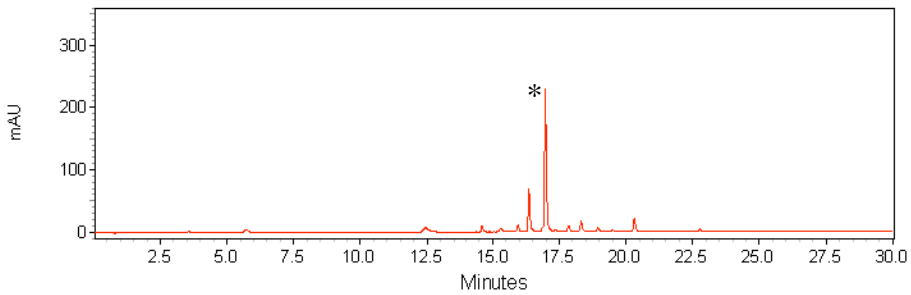


Fig. S5 HPLC trace of product **10a** at 280 nm on aminoethyl-photolinker AM resin. The peaks under the asterisk are the two diastereomers of compound **10a**.

UV calibration curve for model DKP product (10a).

A calibration curve was generated for the model DKP product **10a** using the material described above synthesized on photolinker AM resin. A 2.49 mg portion of the white solid was dissolved in 1:1 H_2O/CH_3CN in a 10 mL volumetric flask to make a 0.64 mM solution. This solution was diluted 1, 2, 5, 10, 20, 50, and 100x with the same solvent mixture. HPLC spectra were obtained for the solutions, and the resulting UV intensities at 280 nm were plotted against the solution concentrations to yield the graph shown in Fig. S6.

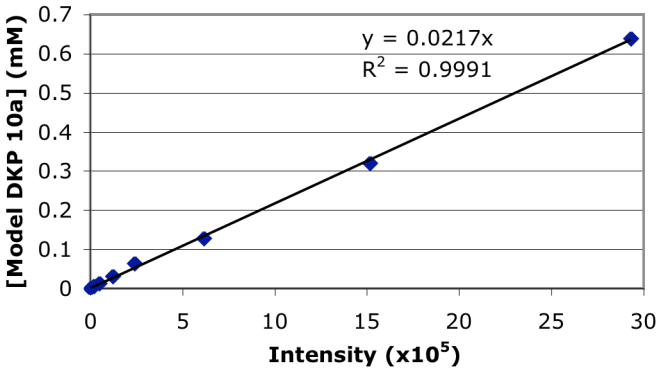


Fig. S6 Calibration curve generated for model DKP product **10a** by HPLC analysis with UW detection at 280 nm.

Synthesis of 24-member DKP macroarray.

Synthesis of Ugi macroarray 6. Neat aldehydes and 2.0 M solutions of carboxylic acids in DMF were mixed in 1:4 volume ratios (~1:1 mole ratio) to make 25 μL. A 4.0 μL aliquot of Millipore water was spotted onto specific locations on amine-derived support **5** (24 spots, 1.2 cm x 1.2 cm grid), followed by a 3.0 μL aliquot of the pre-mixed solution of aldehyde and carboxylic acid and 1.2 μL of the 1-isocyanocyclohexene/hexanes mixture (2:1 mole ratio by ^{1}H NMR). The support was allowed to stand in darkness at 25 °C for 10 min. This procedure was repeated twice with reduced amounts of each reagent (2.0 μL water, 1.2 μL aldehyde/carboxylic acid mixture, and 0.8 μL isocyanide) to minimize contamination between spots due to spreading. The support was then washed by immersion and swirling in DMF, MeOH, DMF (2x) and MeOH (2x). The resulting Ugi macroarray **6** was dried in a vacuum oven at 100 °C overnight.

Synthesis of DKP macroarray 9. Support **6** was cut into 8 pieces, each containing 3 compounds. Each piece was placed in a 70 mL Teflon/PEEK MW reaction vessel. A 22 mL volume of 10% AcCl in MeOH was added to each of these reaction vessels. The vessels were sealed and subjected to MW irradiation (heated to 80 °C over 5 min; held at this temperature for 30 min). The solutions were carefully decanted from the vessels. The support sections were then washed with MeOH (3x) and CH_2Cl_2 (1x) while inside the reaction vessels. After being dried under a stream of N_2 for 30 min, the pieces of support **7** were removed from the vessels and placed on a Teflon watch glass. Aliquots (3.0 μL) of 20% piperidine in DMF were spotted onto the individual spots of supports **7** and allowed to sit at 25 °C for 20 min. This procedure was repeated twice, followed by washing with DMF (3x) and MeOH (3x). The supports were dried

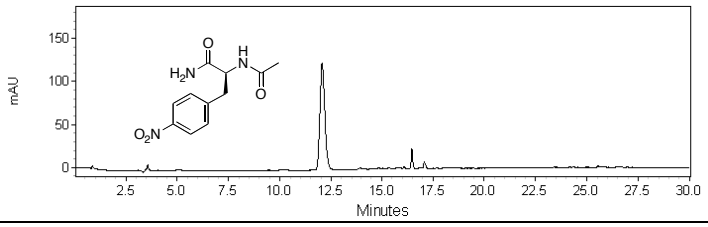
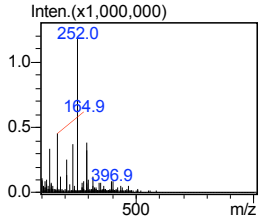
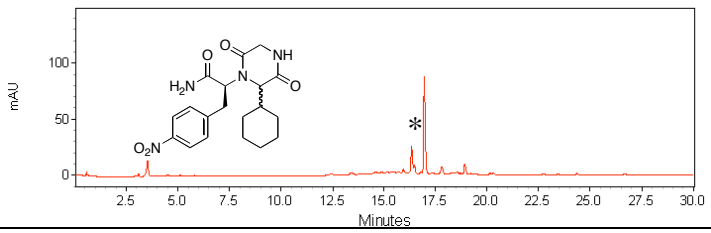
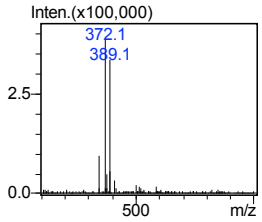
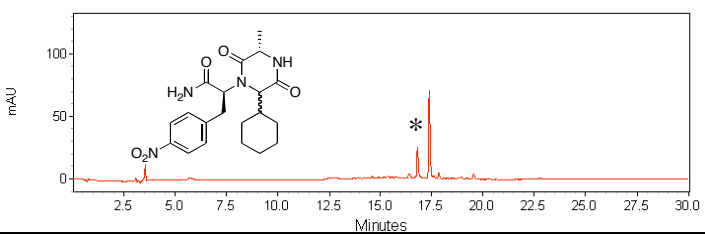
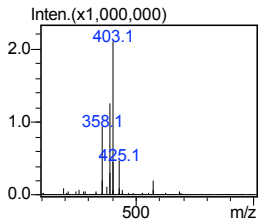
and acetylated using the method described above. The resulting DKP array **9** was subjected to photocleavage as described below.

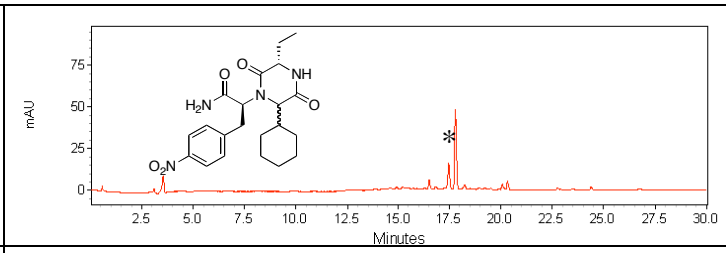
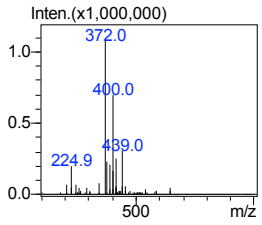
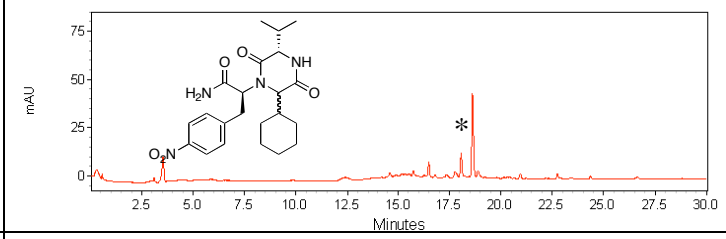
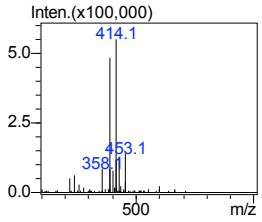
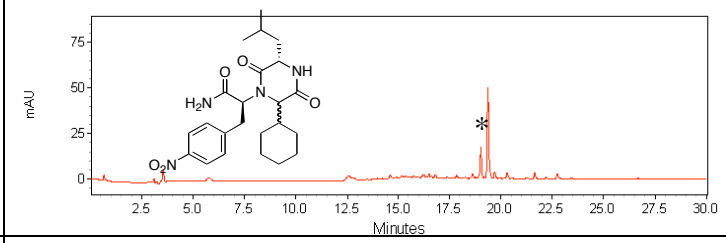
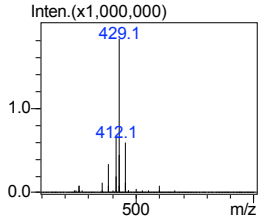
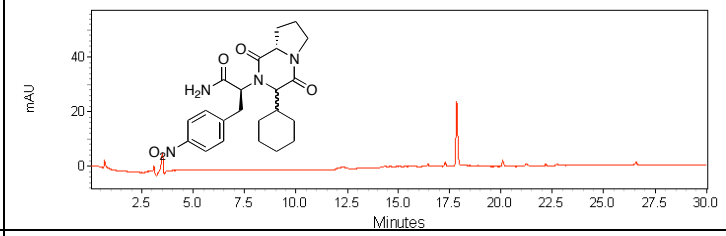
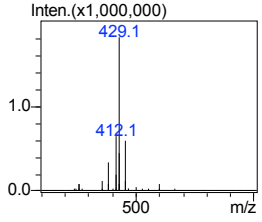
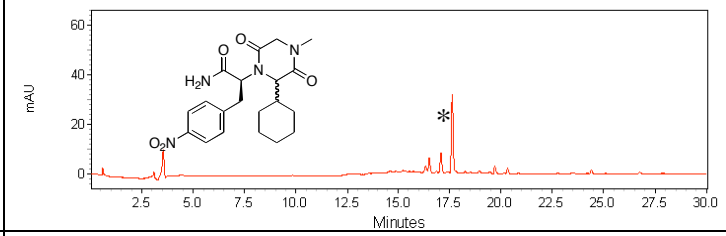
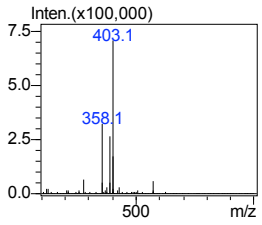
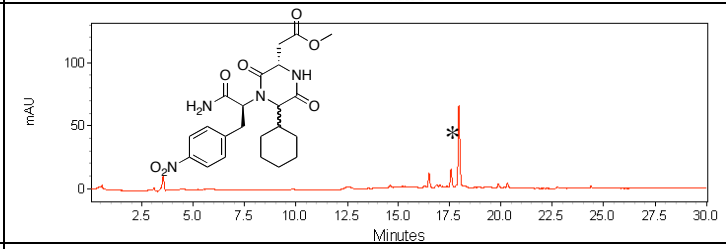
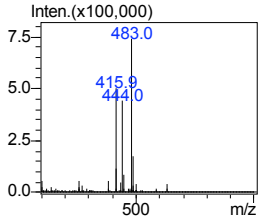
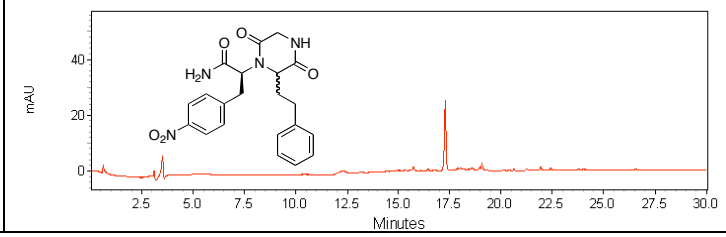
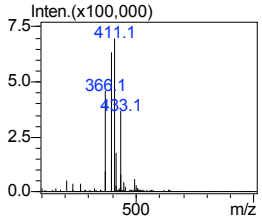
Photocleavage of DKP macroarray 9. Individual spots from DKP macroarray **9** were punched-out and placed in vials containing 0.5 mL of MeOH. Photocleavage was performed and the cleaved compounds (**10**) were isolated as described above for model DKP product **10a**.

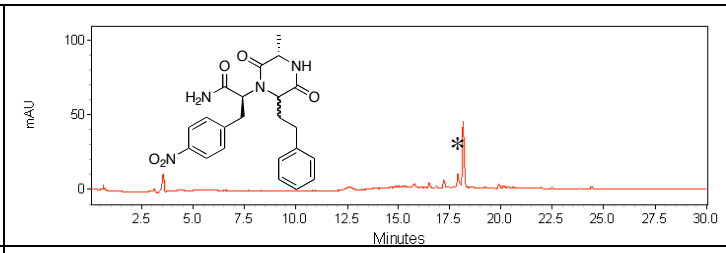
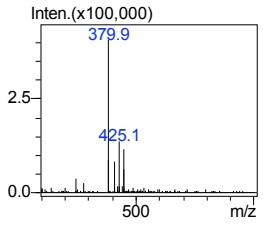
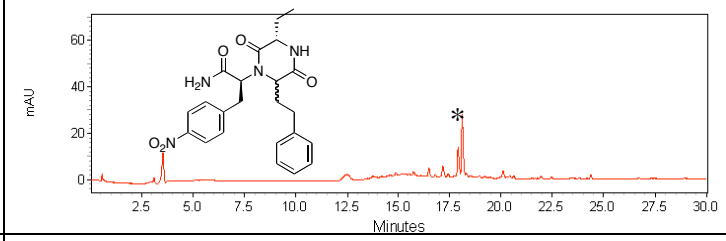
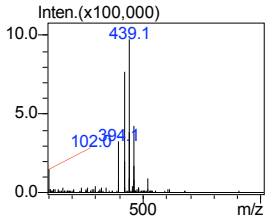
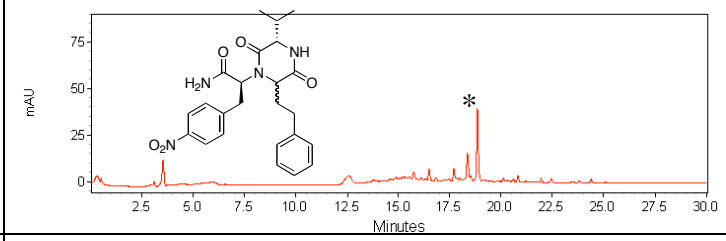
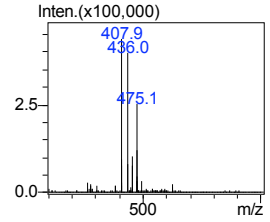
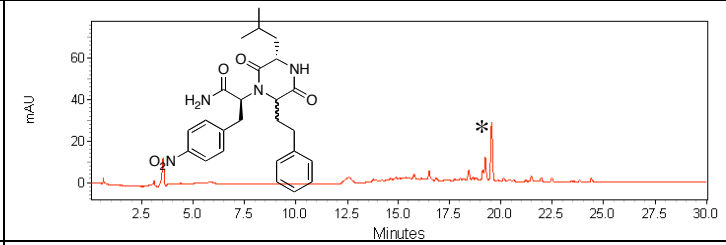
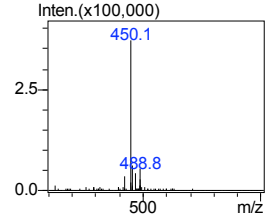
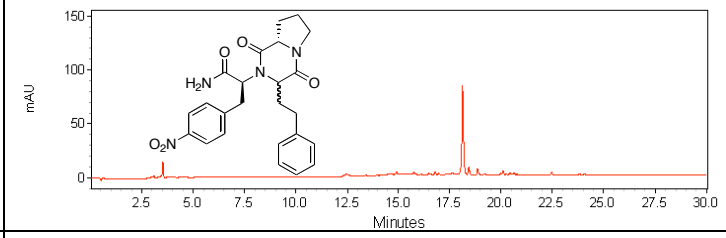
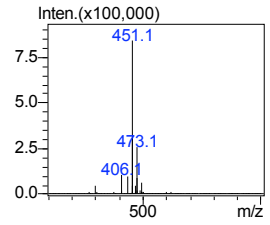
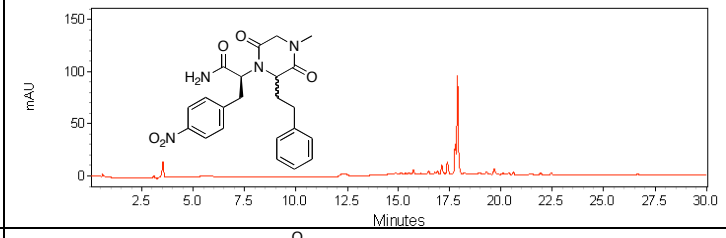
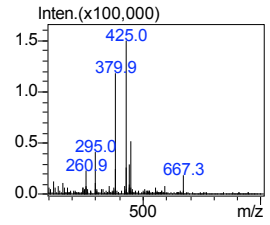
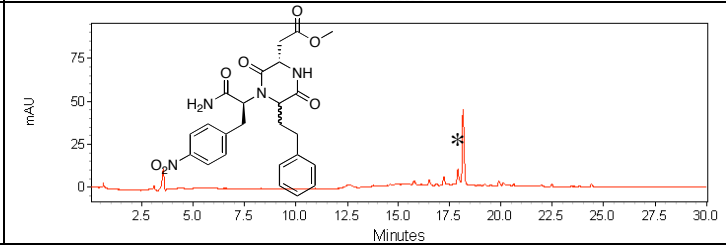
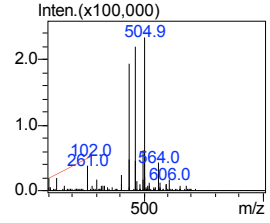
HPLC and LC-MS analysis of DKP products 10. DKPs (**10**) were each dissolved in 100 µL of CH_3CN/H_2O (1:1, v/v), filtered, and analyzed by HPLC and LC-MS. **Method specifics**: HPLC: solvent gradient of 15–95% B over 16.5 min followed by 9.5 min at 95% B. LC: solvent gradient of 15–95% B over 16 min followed by 4 min at 95% B. MS: positive ion mode was used for all samples. UV detection at 280 nm. HPLC and MS data can be found in the following section.

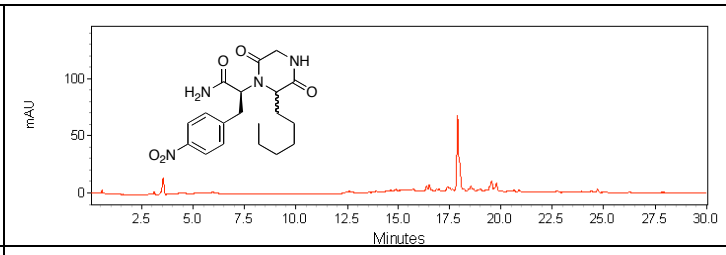
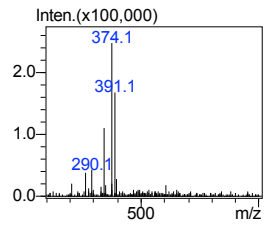
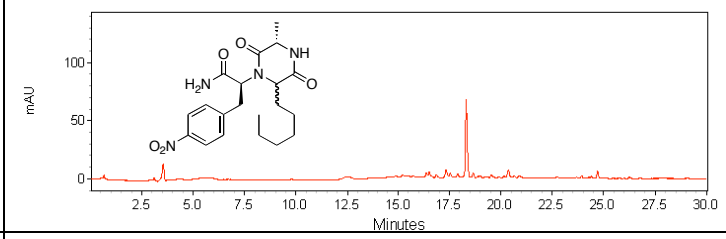
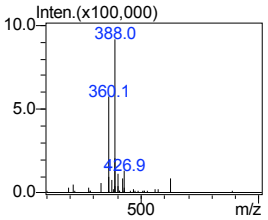
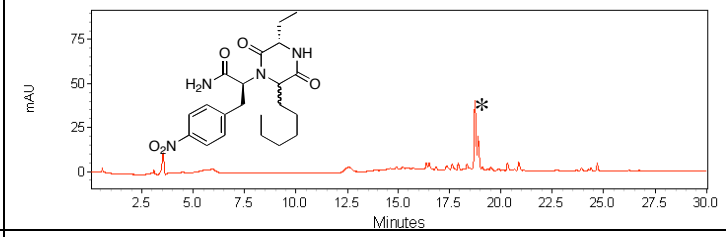
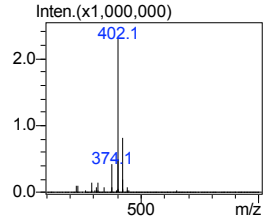
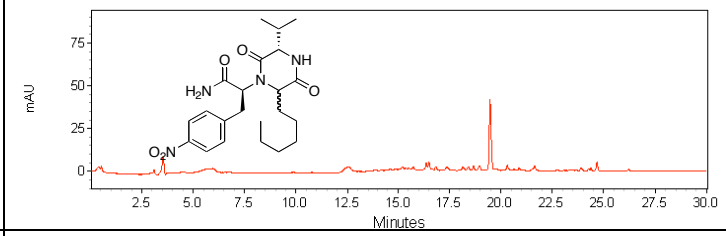
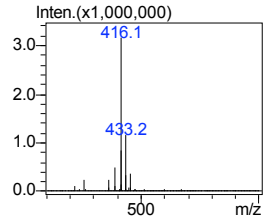
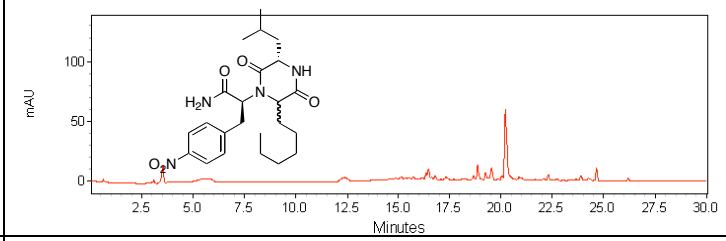
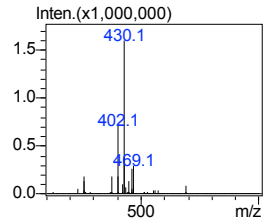
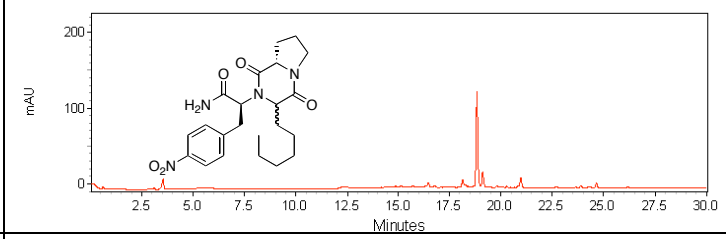
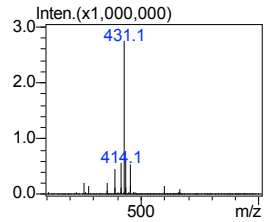
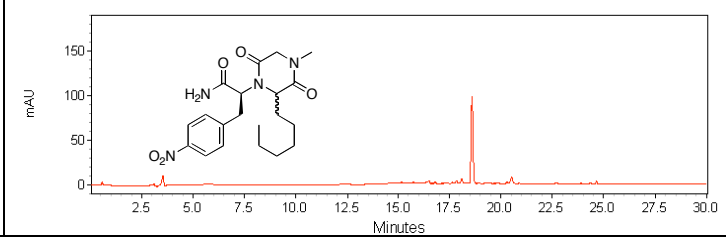
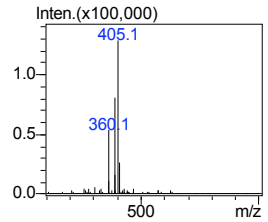
HPLC and MS data for 24-member DKP macroarray.

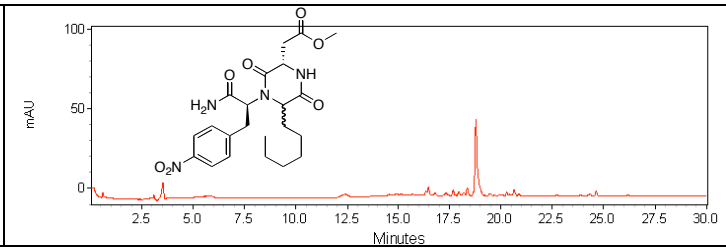
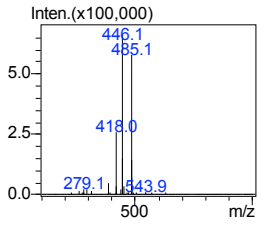
Peaks under an asterisk (*) are diastereomers of the major peak. Purity reported as combined data for both diastereomers.

Sample Exact Mass Purity	**HPLC chromatogram at 280 nm**	**MS of major peak** ESI (positive ion mode)
Ac-Nph-NH_2 **"Starting material amine"** 251.1 95%		
10a **Aa** 388.2 84%		
10b **Ab** 402.2 90%		

10c **Ac** 416.2 86%		
10d **Ad** 430.2 84%		
10e **Ae** 440.2 83%		
10f **Af** 428.2 90%		
10g **Ag** 402.2 80%		
10h **Ah** 460.2 86%		
10i **Ba** 410.2 85%		

10j **Bb** 424.2 74%		
10k **Bc** 438.2 73%		
10l **Bd** 452.2 65%		
10m **Be** 466.2 66%		
10n **Bf** 450.2 82%		
10o **Bg** 424.2 81%		
10p **Bh** 482.2 82%		

10q **Ca** 390.2 80%		
10r **Cb** 404.2 80%		
10s **Cc** 418.2 70%		
10t **Cd** 432.2 58%		
10u **Ce** 446.2 53%		
10v **Cf** 430.2 80%		
10w **Cg** 404.2 91%		

10x **Ch** 462.2 81%		

References and notes.

1. For further information about this microwave reactor, see: http://www.milestonesci.com/

2. We previously reported a version of this derivatization protocol. See: Q. Lin, J. C. O'Neill and H. E. Blackwell, *Org. Lett.*, 2005, **7**, 4455-4458.

3. For details of the Kaiser test, see: E. Kaiser, R. L. Colescot, C. D. Bossinge and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595-598.

4. 1-Isocyanocyclohexene was prepared according to the method of Keating *et al.* and stored as a mixture (2:1 mole ratio by ^{1}H NMR) with hexanes at -10 °C. See: T. A. Keating and R. W. Armstrong, *J. Org. Chem.*, 1996, **61**, 8935-8939.