Supporting Information

# Microwave Assisted Synthesis of Py-Im Polyamides

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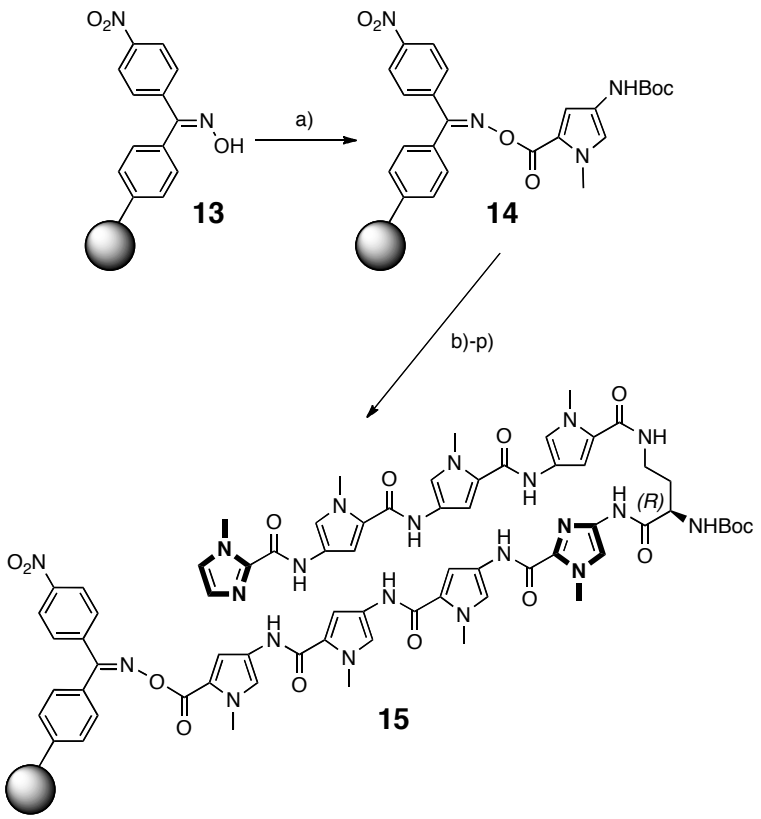
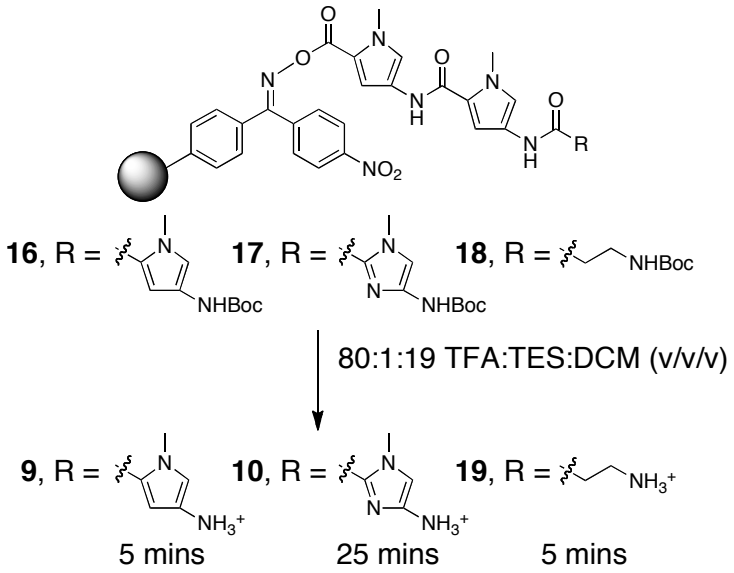
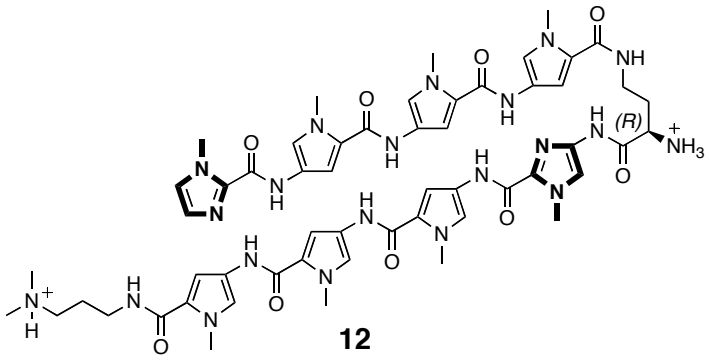


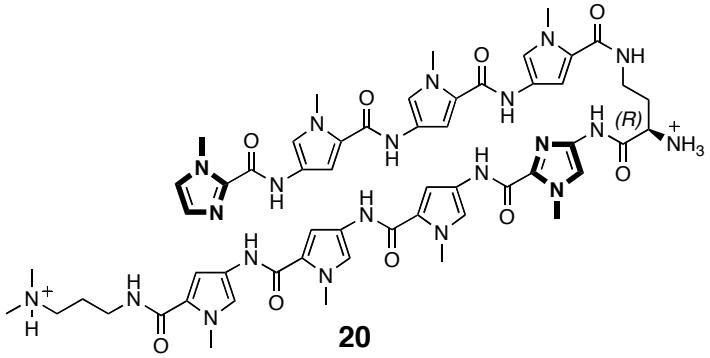

**Supporting Figure 1**. a) Boc-Py-OBt (**1**), DIEA, DMF, 80 ºC, microwave, 3 h; b) 80:1:19 TFA:triethylsilane: $CH_2Cl_2$ (v/v/v), 5 mins, RT; c) Boc-Py-OBt, DIEA, DMF, 60 ºC, microwave, 5 mins; d) $Ac_2O$, DIEA, DMF, RT, 10 min; e) repeat b)-d); f) 80:1:19 TFA:triethylsilane:$CH_2Cl_2$, 5 mins, RT; g) Boc-Im-OH, DIEA, PyBOP, DMF, 60 ºC, microwave, 5 mins; h) $Ac_2O$, DIEA, DMF, RT, 10 min; i) 80:1:19 TFA:triethylsilane:$CH_2Cl_2$, 25 mins, RT; j) Fmoc-D-Dab(Boc)-OH, DIEA, PyBOP, DMF, 60 ºC, microwave, 25 mins; k) $Ac_2O$, DIEA, DMF, RT, 10 min; l) repeat b)-c) three times; m) 80:1:19 TFA:triethylsilane:$CH_2Cl_2$, 5 mins, RT; n) $ImCCl_3$, DIEA, DMF, 60 ºC, microwave, 5 min; o) 20% piperidine / DMF, 60 ºC, microwave, 3 mins; p) $Boc_2O$, DIEA, DMF, 60 ºC, 3 mins




**Supporting Figure 2.** Trifluoroacetic acid deprotection conditions for resins **16–18**.

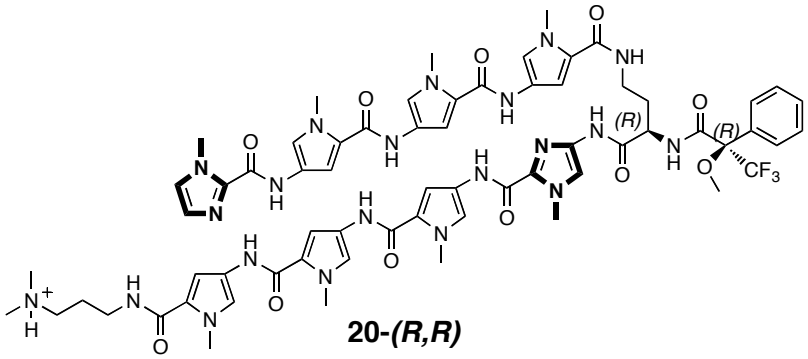


**12**



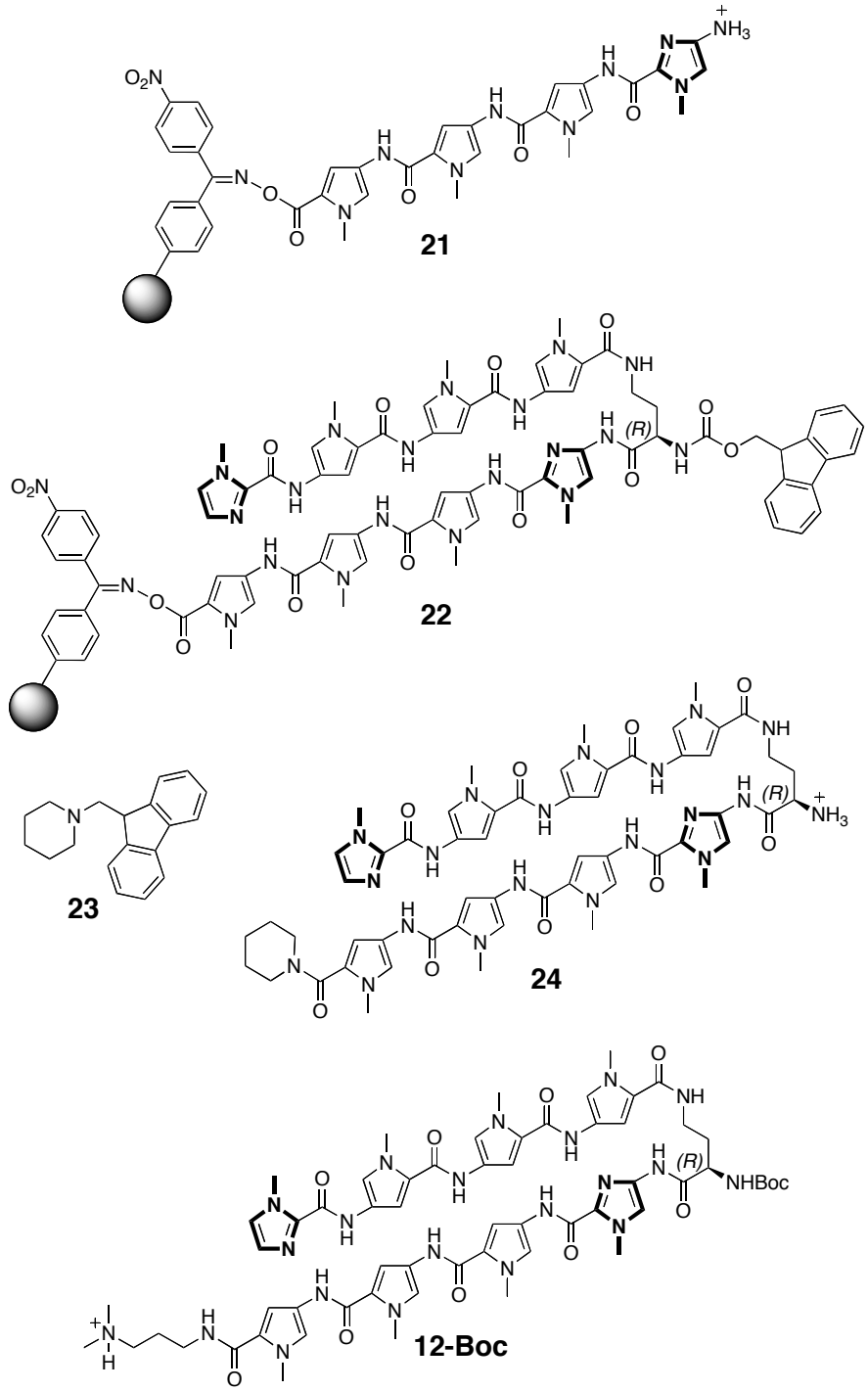
**20**

synthesized by traditional methods

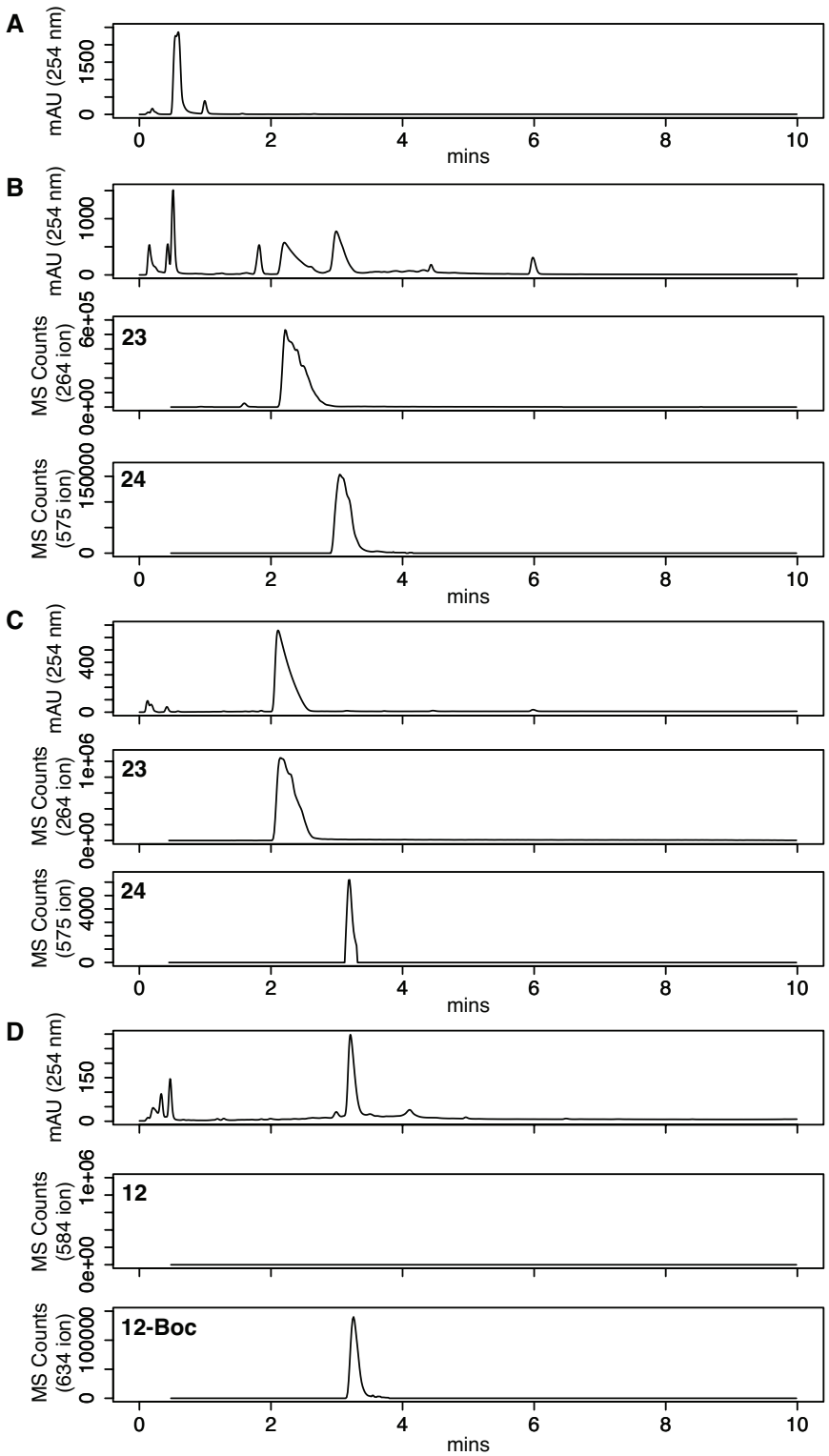


**20-*(R,R)***

**Supporting Figure 3.** Polyamide **12** was utilized to synthesize Mosher Amide conjugates **12-*(R,R)*** and **12-*(R,S)***. Polyamide **20** was synthesized by solid phase peptide synthesis on Kaiser oxime resin, using methods described in Belitsky, et al.[1] It was prepared as a reference for comparing d.e. of **20-*(R,R)*** to that of **12-*(R,R)***.




**Supporting Figure 4.** Resin **21** was prepared *en route* to **15**. Resin **22** was prepared prior to exchange of Fmoc for a Boc protecting group. Compound **23** is the Fmoc-piperidine byproduct from Fmoc deprotection using 20% piperidine / DMF. Polyamide **24** was a byproduct of Fmoc deprotection, present in approximately 0.4% yield. Polyamide **12-Boc** was synthesized to evaluate whether full Fmoc removal had occurred prior to resin cleavage with Dp. Presence of **12-Boc** and absence of **12** would demonstrate full Fmoc removal by 20% piperidine / DMF.




**Supporting Figure 5.** UPLC-MS data for stability of oxime resin to cleavage. a) Resin **21** does not appear to cleave in the presence of HOBt, DIEA, and DMF at 60 ºC in the microwave for 25 min. b) UPLC-MS data for extensive cleavage of resin **22** using 95% piperidine / DMF in microwave at 80 ºC for 20 min. Two products of significance form: **23** (singly charged ion) and **24** (doubly charged ion). c) UPLC-MS data for 30 s exposure of resin **22** to 20% piperidine / DMF in the microwave at 60 ºC. d) UPLC-MS data for products resulting from Boc protection at the end of the reaction from part c). Only polyamide **12-Boc**, and not polyamide **12**, forms.

*Note:* All compound and table numberings refer to those in the primary text. Supporting information compounds contain numbers greater than those in the primary text.

## *List of Reagents and Suppliers*

| Reagent | Vendor |
|---|---|
| *N,N*-dimethylformamide (4L bottle) | EMD Biosciences |
| Oxime resin LL (100-200 mesh) | EMD Biosciences |
| PyBOP | EMD Biosciences |
| HPLC-grade dichloromethane | Fluka |
| Anhydrous diethyl ether | Fluka |
| Z-β-Dab(Boc)-OH | Fluka |
| (*R*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride | Fluka |
| (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride | Fluka |
| Anhydrous methanol | Sigma-Aldrich |
| HPLC-grade acetonitrile | Sigma-Aldrich |
| Trifluoroacetic acid (purified by redistillation, for protein sequencing) | Sigma-Aldrich |
| Trifluoroacetic acid (*ReagentPlus*, 99%) | Sigma-Aldrich |
| Triethylsilane (99%) | Sigma-Aldrich |
| *N,N*-diisopropylethylamine (99%, redistilled) | Sigma-Aldrich |
| 3,3'-Diamino-*N*-methyl-dipropylamine (96%) | Sigma-Aldrich |
| 3-(Dimethylamino)-1-propylamine | Sigma-Aldrich |
| *N*-butylamine | Sigma-Aldrich |
| Piperidine (≥99%, redistilled) | Sigma-Aldrich |
| Di-*tert*-butyl dicarbonate (*ReagentPlus*, ≥99%) | Sigma-Aldrich |
| PyAOP | Oakwood Chemicals |
| Boc-β-Ala-OH | Peptides International |
| Boc-γ-Abu-OH | Peptides International |
| Fmoc-D-Dab(Boc)-OH | Peptides International |

Boc-Py-OBt, Boc-Py-OH, Boc-Im-OH, Im-$CCl_3$, and Ct-OH were prepared as described previously.[2]

## *List of Instruments*

| | |
|---|---|
| Microwave | Biotage Initiator Eight |
| Analytical HPLC | Beckman Gold |
| Semi-preparative HPLC | Agilent 1200 |
| Analytical UPLC | Agilent 1290 |
| MALDI-TOF MS | Voyager DE Pro |

## *Resin Swelling, Washing, and Deswelling Procedures*

The coupling chemistries explored herein necessitate *N*,*N*-dimethylformamide (DMF), dichloromethane (DCM), and methanol (MeOH) wash steps. The reactions are performed on 10 mg, 100 mg, and 1 g resin scales. At the 10 mg scale, the resin was washed three times with 1 mL of each solvent (3 x 1 mL). At the 100 mg scale, each wash is 3 x 2 mL, and at the 1 g scale, each wash is 3 x 15 mL. For each wash, the solid-phase synthesis vessel was agitated sufficiently to disperse the resin beads throughout the solvent. Additionally, when switching solvents so as to swell or deswell the resin, the resin was allowed to soak in the solvent until a size change in the resin was observed. Aside from these concerns, the length of each wash is not critical.

## *Loading Boc-Py-OBt*

A 10–20 mL Biotage microwave synthesis vessel was charged with dry, deswollen Kaiser oxime resin (**13**, 1.2 g, 1.02 mmol) (Supp. Fig. 1). Boc-Py-OBt (**1**, 1.46 g, 4.08 mmol) was dissolved into 10 mL DMF and then added to **13**. Diisopropylethylamine (DIEA, 711 μL, 4.08 mmol) and a stir bar were added prior to sealing the microwave vessel. The vessel was placed into a Biotage Initiator Eight microwave synthesis machine and allowed to react for 3 h at 80 ºC, 650 RPM, very high absorbance, FHT on. The reaction vessel was opened, and resin was transferred as a slurry to a manual solid phase peptide synthesis vessel to drain the reagents from the resin. The resin was washed with DMF and DCM, and then deswollen using MeOH. The deswollen resin was dried *in vacuo* overnight, affording 1.56 g of **14**, the loaded resin.

The monomer Boc-Im-OH (**2**) can be loaded onto Kaiser oxime resin using the same conditions as for **1** but with the addition of 4 equivalents of PyBOP and the use of 8 equivalents of DIEA. Subsequent couplings cleave the monomer from resin, rendering this loading impractical. For applications necessitating a *C*-terminal Im monomer, the hydrazine resin is a reasonable alternative[3] and can function using microwave chemistry.

## *Optimization of Deprotection Time*

Resins **16**, **17**, and **18** (10 mg each) were swollen in manual solid phase synthesis vessels by washing with DMF and then $CH_2Cl_2$ (DCM). The 80:1:19 TFA:triethylsilane (TES):DCM mix (1 mL) was added to the synthesis vessel and allowed to react for 5 min to 30 min, in 5 min intervals. At each time point, the resin was washed with DCM, DMF, and then DCM. Use of *ReagentPlus*® trifluoroacetic acid (Sigma-Aldrich Cat. No. T6508) afforded only 80-90% deprotection over 25 min. Exposure of resin to *ReagentPlus*® TFA for longer periods did not enable greater than 80-90% deprotection. Use of protein sequencing grade, redistilled trifluoroacetic acid is essential to achieve full deprotection of the Boc-protected *N*-methylimidazole monomer.

Over 99% deprotection, as determined by analytical HPLC, occurred in 5 min for **16→9** and **18→19**. The acid deprotection of Im monomer is known to take longer, and 25 min was sufficient for its deprotection (**17→10**) (Supp. Fig. 2).

## *Screening of Coupling Times*

Coupling times were screened using 20 mg (18.6 μmol) portions of dried, deswollen **9** or **10**. Tables 1 and 2 of the primary text provide number of equivalents of material utilized and final concentrations of the monomer with respect to total solution volume. An example for coupling of 20 mg of resin **10** to monomer **1-OH** in Table 2:

Monomer **1-OH** (17.9 mg, 74 μmol), PyAOP (38.6 mg, 74 μmol), DIEA (19.5 μL, 14.5 mg, 112 μmol), and DMF (227 μL) were added to a small glass vial and stirred to dissolve the monomer. Resin **10**, a stir bar, and the dissolved monomer **1-OH** cocktail were added to a 2 mL Biotage microwave synthesis vessel. The vessel was sealed, placed into the microwave synthesis machine, and allowed to react for 60 mins at 80 ºC (FHT on, very high absorbance).

All other conditions in Tables 1 and 2 of the primary text are conducted analogously to that described in the example above, substituting appropriate numbers of equivalents, activating reagents, and coupling times as necessary. All reactions, except those denoted by ‡ were run at 60 ºC. Those containing ‡ were run at 80 ºC. Concentrations of monomers delineated in tables 1 and 2 should be increased if using pre-swollen resin. In the case of the resin utilized here (EMD Biosciences Oxime resin LL 100-200 mesh Cat No. 855089), 0.4 M concentrations were sufficiently high to approximate the same final concentration of monomer.

## *Detailed Synthesis of Polyamide 11*

Kaiser oxime resin (100 mg, 93 μmol) and Boc-Py-OBt (133 mg, 372 μmol, 4 eq.) were added to a 2 mL microwave synthesis vessel, along with a stir bar (Supp. Fig. 1). DMF (1.24 mL) was added, and the mixture stirred until the Boc-Py-OBt was dissolved. DIEA (64.8 μL, 372 μmol, 4 eq.) was added; the microwave synthesis vessel was then capped and heated in the microwave for 3 h (80 ºC, 650 RPM, FHT on, very high absorbance). A manual solid-phase synthesis vessel was treated with Sigmacote and rinsed thoroughly; the reaction mixture was then transferred as a slurry to this vessel via a pipettor. The resin was rinsed with DMF, DCM, and MeOH (according to the rinse procedure described earlier), then reswollen with DMF. An aliquot of resin was removed and cleaved for HPLC analysis.

The resin was rinsed with DCM. The 80:1:19 TFA:TES:DCM deprotection cocktail (2 mL) was added to the resin, and the solid-phase synthesis vessel placed on a rocker at room temperature for 5 min. The TFA solution was then drained, and the resin rinsed with DCM, DMF, and MeOH. The resin was reswollen with DMF, and an aliquot was taken for HPLC analysis. In a separate vial, Boc-Py-OBt (133 mg, 372 μmol, 4 eq.) and DIEA (32.4 μL, 186 μmol, 2 eq.) were dissolved in DMF (930 μL), and the solution was added to the resin. This mixture, along with a magnetic stir bar, was transferred to the microwave reaction vessel and heated for 5 min (60 ºC, 650 RPM, FHT on, very high absorbance). The mixture was transferred back to the solid-phase synthesis vessel, and the resin rinsed with DMF, DCM, and MeOH. The resin was reswollen with DMF, and an aliquot was taken for HPLC analysis. DMF (1.8 mL), DIEA (400 μL), and acetic anhydride (200 μL) were mixed in a vial, and the mixture added to the resin. The resin was agitated on the rocker for 10 min at room temperature, then drained and rinsed with DMF and DCM.

The above procedure was repeated to attach another Py monomer.

The above procedure was repeated to attach an Im monomer, except that the mixture used for the coupling reaction comprised Boc-Im-OH (89.7 mg, 372 μmol, 4 eq.), PyBOP (194 mg, 372 μmol, 4 eq.), and DIEA (97.2 μL, 558 μmol, 6 eq.) dissolved in DMF (930 μL).

The above procedure was again used to attach the Fmoc-D-Dab(Boc)-OH turn monomer, with the following modifications: the resin was allowed to react with the deprotection solution for 25 min instead of 5 min, the coupling mixture comprised Fmoc-D-Dab(Boc)-OH (164 mg, 372 μmol, 4 eq.), PyBOP (194 mg, 372 μmol, 4 eq.), and DIEA (97.2 μL, 558 μmol, 6 eq.) dissolved in DMF (930 μL), and the coupling time was extended to 25 min.

Following the addition of the turn, a slightly abbreviated procedure was used that omitted the acetyl capping step. The resin was rinsed with DCM, then treated with the deprotection cocktail for 5 min. The TFA solution was then drained, and the resin rinsed with DCM, DMF, and MeOH. The resin was reswollen with DMF, and an aliquot was taken for HPLC analysis. In a separate vial, Boc-Py-OBt (133 mg, 372 μmol, 4 eq.) and DIEA (32.4 μL, 186 μmol, 2 eq.) were dissolved in DMF (930 μL), and the solution was added to the resin. This mixture, along with a magnetic stir bar, was transferred to the microwave reaction vessel and heated for 5 min (60 ºC, 650 RPM, FHT on, very high absorbance). The mixture was transferred back to the solid-phase synthesis vessel, and the resin rinsed with DMF, DCM, and MeOH. The resin was reswollen with DMF, and an aliquot was taken for HPLC analysis.

The above procedure was repeated two more times, to attach a further two Py monomers.

To attach the terminal Im monomer, the procedure was repeated again, with the Boc-Py-OBt replaced with Im-$CCl_3$ (84.6 mg, 372 μmol, 4 eq.).

Piperidine (20% in DMF, 1 mL) was allowed to react with the resin in the microwave for 3 mins (60 ºC, 650 RPM, FHT on, very high absorbance). The resin was then returned to the solid phase synthesis vessel and rinsed with DMF, DCM, and MeOH, followed by reswelling in DMF. The treatment with piperidine was repeated to ensure full deprotection. $Boc_2O$ (203 mg, 930 μmol, 10 eq.) and DIEA (48.6 μL, 279 μmol, 3 eq.) were dissolved in DMF (1.86 mL) and added to the resin; the mixture was then transferred to the microwave synthesis vessel and microwaved for 3 mins (60 ºC, 650 RPM, FHT on, very high absorbance), affording resin **15**. Resin **15** was returned to the solid-phase synthesis vessel, drained, and rinsed with DMF, DCM, and MeOH before reswelling in DMF and taking an aliquot for HPLC analysis. The resin was then deswollen in MeOH and dried in vacuo.

For cleavage, an aliquot of 10 percent of the dry resin (**15**, 22 mg) was added to a solid-phase synthesis vessel and swollen with DMF. 3,3'-diamino-*N*-methyl-dipropylamine (500 μL) was added to the swollen resin, which was then transferred to a microwave synthesis vessel and heated for 10 mins (60 ºC, 650 RPM, FHT on, very high absorbance). The reaction mixture was transferred back to the solid-phase synthesis vessel, and a further 500 μL DMF was used to rinse the microwave synthesis vessel. The liquid (crude **11**) was drained into a conical tube; resin **15** was washed with a further 2 x 1 mL DMF, which was also collected in the conical tube. Diethyl ether (30 mL) was added to the tube, which was then placed in a freezer at -20 ºC for 30 min. The tube was then centrifuged at 4200 RPM at 4 ºC for 30 min, and the supernatant removed. The precipitate (crude **11**) was suspended in 5 mL of 0.1% TFA/$H_2O$ solution, and 1.0 N HCl in $H_2O$ was added at 0 ºC to reduce the pH to approximately 3. This solution was then loaded directly onto the preparative HPLC.

Following the HPLC run, the UV/Vis trace was examined, and the fractions corresponding to the major peak were further analyzed by analytical HPLC. Fractions that showed only clean polyamide **11** with no contaminants were combined in a Falcon conical tube, frozen in liquid nitrogen, and lyophilized to dryness, affording a fluffy pale yellow solid. This was centrifuged into a pellet, then dissolved in Gibco UltraPure distilled water. An aliquot of this solution was serially diluted and analyzed by UV/Vis spectroscopy to quantify the yield (5.2 μmol, 56 %) of the polyamide (using a molar extinction coefficient of 69,500 $M^{-1} \cdot cm^{-1}$). A second aliquot was analyzed by MALDI-TOF mass spectroscopy to confirm the identity of the product (expected $[M+H]^+$: 1309.6, observed $[M+H]^+$: 1309.7).

Compound **12** was prepared as described for **11**, but no Boc-protection was performed after Fmoc removal. The resin sample was cleaved using dimethylaminopropylamine. Compound **20** was synthesized using previously described methods.[1,4] As with the synthesis of **12**, for compound **20** no Boc-protection was performed at the end of the synthesis. Cleavage was accomplished using dimethylaminopropylamine.

## *Notes on Maximizing Yield*

When an aliquot of resin-bound **15** was cleaved to give **11** and purified using a reversed-phase HPLC column that had not been thoroughly cleaned prior to use (but was in other respects identical to the one used to achieve 56% yield), isolated yield dropped to 44%. Additionally, larger aliquots (on the order of half of the cleavage eluent from a 100 mg resin scale batch) purified in one HPLC run resulted in a reduced yield of 46%. It seems likely, therefore, that a significant amount of polyamide may be lost during purification, depending on the precise column conditions and loading.

Polyamide **11** was also synthesized on a smaller scale, starting with 20 mg of Kaiser oxime resin (19 μmol). The methodology used was the same as described above; however, we were unable to achieve yields higher than 21%. This may be due to less efficient stirring in the smaller microwave reaction vessel, or it may be that the inevitable loss of small quantities of resin with each analytical HPLC aliquot and each transfer from solid-phase synthesis vessel to microwave vessel and back consumes a higher fraction of the resin on the smaller scale than on the larger. Nevertheless, the amount of polyamide generated on this scale (4.0 μmol) is sufficient for initial biological experiments, so the lower yield may be acceptable in light of the savings in material cost when building a library of polyamides to test in cell culture experiments.

## *Stability of Oxime Resin to General Coupling Conditions*

Because of the labile oxime-ester bond linking the growing Py-Im polyamide to the solid support, we checked whether the microwave conditions themselves would facilitate cleavage from resin. To a 2 mL Biotage microwave vessel charged with 5 mg sample of resin **21** (Supp. Fig. 4) were added DMF (500 μL), DIEA (20 μL, 19.2 mg, 15 μmol), HOBt (28 mg, 207 μmol), and a stirbar. The vessel was sealed and allowed to react at 60 ºC, FHT on, very high absorbance in the microwave for 25 mins. A sample of the supernatant (100 μL) was diluted using 0.1 % TFA / $H_2O$ (400 μL), and a 2 μL sample was injected for analysis by UPLC-MS. No cleavage products were observed containing a characteristic absorbance for a polyamide or a mass corresponding to products related to **21** (Supp Fig. 5A).

## *Stability of Oxime Resin to Fmoc Deprotection Conditions*

Because of the nucleophilicity of piperidine, we tested whether the Fmoc removal conditions would cause nucleophilic cleavage of the Py-Im polyamide from the solid support. As a control reaction to demonstrate extensive cleavage by piperidine, a 2 mL microwave vessel was charged with resin **22** (5 mg), piperidine (480 μL), and DMF (20 μL) (Supp. Fig. 4). The vessel was sealed and allowed to react at 80 ºC, FHT on, very high absorbance, for 20 min. The supernatant of the reaction (100 μL) was diluted using 0.1 % TFA / $H_2O$ (400 μL), and a 2 μL sample was injected for analysis by UPLC-MS. At a monitored absorbance of 254 nm, the areas of the peaks corresponding to **23** and **24** were 8699 and 8307, respectively (Supp. Fig. 5B).

To a 2 mL Biotage microwave vessel charged with 50 mg resin **22** (Supp. Fig. 4) were added DMF (800 μL) and piperidine (200 μL). The vessel was sealed and allowed to react at 60 ºC, FHT on, very high absorbance in the microwave for 30 s. A sample of the supernatant (50 μL) was diluted using 0.1 % TFA / $H_2O$ (450 μL; 1.0 N HCl was added dropwise to reduce the pH to solubilize material), and a 2 μL sample was injected for analysis by UPLC-MS. At a monitored absorbance of 254 nm, the areas of the peaks corresponding to **23** and **24** were 9621 and 35, respectively (Supp. Fig. 5C).

Because resin cleavage with Dp will also cleave any remaining Fmoc on the polyamide, we chose to Boc protect the resin resulting from the previous 20% piperidine / DMF treatment. The Boc protection will then give rise to only **12** or **12-Boc** upon Dp cleavage, enabling the close approximation of the extent of Fmoc removal. The remaining supernatant and resin were transferred to a manual solid phase synthesis vessel for washing with DMF, DCM, MeOH, and then DMF. $Boc_2O$ (100 mg), DIEA (40 μL), and DMF (1 mL) were combined with the resin. The resin mixture and a stirbar were transferred to a microwave vessel, and the vessel was sealed. The reaction proceeded at 60 ºC, FHT on, very high absorbance in the microwave for 5 min. The resin was transferred into a manual synthesis vessel, and an aliquot was removed for Dp cleavage and UPLC-MS analysis. We observed complete deprotection of the Fmoc under these conditions as measured by UPLC-MS (absence of compound **12** and presence of **12-Boc;** Supp. Fig. 5D).

We calculate that approximately 0.4% of the Py-Im polyamide on resin was nucleophilically cleaved from resin during 30 s, microwave-assisted Fmoc deprotection:

$$\%_{cleavage} = \frac{\frac{area_{\mathbf{24}_{Sample}}}{area_{\mathbf{23}_{Sample}}}}{\frac{area_{\mathbf{24}_{Ctl}}}{area_{\mathbf{23}_{Ctl}}}} \qquad \text{Equation 1}$$

A one minute deprotection time in the microwave resulted in approximately 1.2% nucleophilic cleavage, and a three minute time resulted in approximately 3.4% nucleophilic cleavage.

### *Alternate HPLC Sample Preparation Method*

In addition to the ether precipitation method described above, another method was used to prepare samples of cleaved polyamide for purification by HPLC. In this method, the cleavage eluent was acidified to pH ~ 3 using 1.0N HCl in $H_2O$ at 0 ºC, then loaded onto a Waters C18 Sep-Pak. 0.1% TFA was used to elute the excess cleavage agent. The polyamide was eluted from the Sep-Pak with CH3CN, diluted with 0.1% TFA, lyophilized, and purified by semi-preparative HPLC. Following purification, this method gave 52% yield, slightly lower than the yield obtained by the ether precipitation method.

### *Preparation of Mosher Amides*

Polyamide **12** or **20** (100 nmol) was dissolved in anhydrous acetonitrile (500 µL), to which was added anhydrous DIEA (174 nL, 1 µmol, 10 eq.) and either the (*R*) or (*S*) Mosher acid chloride (93.5 nL, 500 nmol, 5 eq.). The DIEA and acid chloride were added as solutions in anhydrous acetonitrile to facilitate handling such small quantities of reagent. The mixture was gently agitated for 30 min at room temperature, then diluted in 0.05 % TFA/$H_2O$. Conversion to the amide is virtually quantitative by analytical HPLC.

## *Analytical HPLC Characterization of Synthesis Intermediates*

*Column*: Phenomenex Gemini NX C18, 4.6 mm x 50 mm, 5 µm particle size, 110 Å pore size
*Instrument*: Beckman Gold semi-preparative HPLC system
*Gradient*: 5-95% $CH_3CN$ in 0.1% TFA/$H_2O$ over 7 mins at 1 mL / min.

A minimal aliquot of DMF or DCM-washed and drained resin was removed from the peptide synthesis vessel using the tip of a Pasteur pipette and transferred to a microcentrifuge tube. 3-(Dimethylamino)-1-propylamine (Dp; 150 µL) was added to the microcentrifuge tube containing resin. If the resin-bound polyamide chain contained fewer than three monomers, *N*-butylamine was used in place of Dp to avoid formation of low molecular weight, doubly-cationic species, which have insufficient retention on the reversed-phase HPLC column. The microcentrifuge tube was placed on a 95 ºC heat block for 5 mins. Trifluoroacetic acid (TFA; 0.1% in $H_2O$, 600 µL) was added to the microcentrifuge tube; the tube was vortexed and centrifuged briefly. A 200 µL portion of the liquid phase in the microcentrifuge tube was utilized for HPLC. Approximate yields are calculated by integrating the areas of starting material ($area_{sm}$) and product peaks ($area_{pdt}$) and calculating the ratio $area_{pdt}$ / ($area_{pdt}$ + $area_{sm}$) at their local $\lambda_{max}$, near 310 nm.

## *Analytical UPLC Conditions for Separating Mosher Amide Diastereomers*

*Column*: Agilent 2.1 mm x 150 mm C18, 1.9 µm particle size
*Instrument*: Agilent 1290 UPLC System
*Gradient*: 28 – 32% $CH_3CN$ in 0.05% TFA/$H_2O$ over 36 mins at 0.6 mL / min.

## *Analytical UPLC-MS Conditions*

*Column*: Agilent 2.1 mm x 50 mm C18, 1.9 µm particle size
*Instrument*: Agilent 1290 UPLC System
*Gradient*: 5 – 95% $CH_3CN$ in 0.1 N AcOH/$H_2O$ over 10 mins at 1.0 mL / min.

## *Semi-Preparative HPLC Conditions*

*Column*: Phenomenex Gemini C18, 21 mm x 250 mm, 5 µm particle size, 110 Å pore size
*Instrument*: Agilent 1200 HPLC System
*Gradient*: 19-23% $CH_3CN$ in 0.1% TFA/$H_2O$ over 60 mins at 10 mL / min.

## *Mass Spectral Data of Compounds*

MALDI-TOF MS data

**11** ($C_{62}H_{81}N_{22}O_{11}^{+}$): expected $[M+H]^{+}$: 1309.6, observed $[M+H]^{+}$: 1309.7

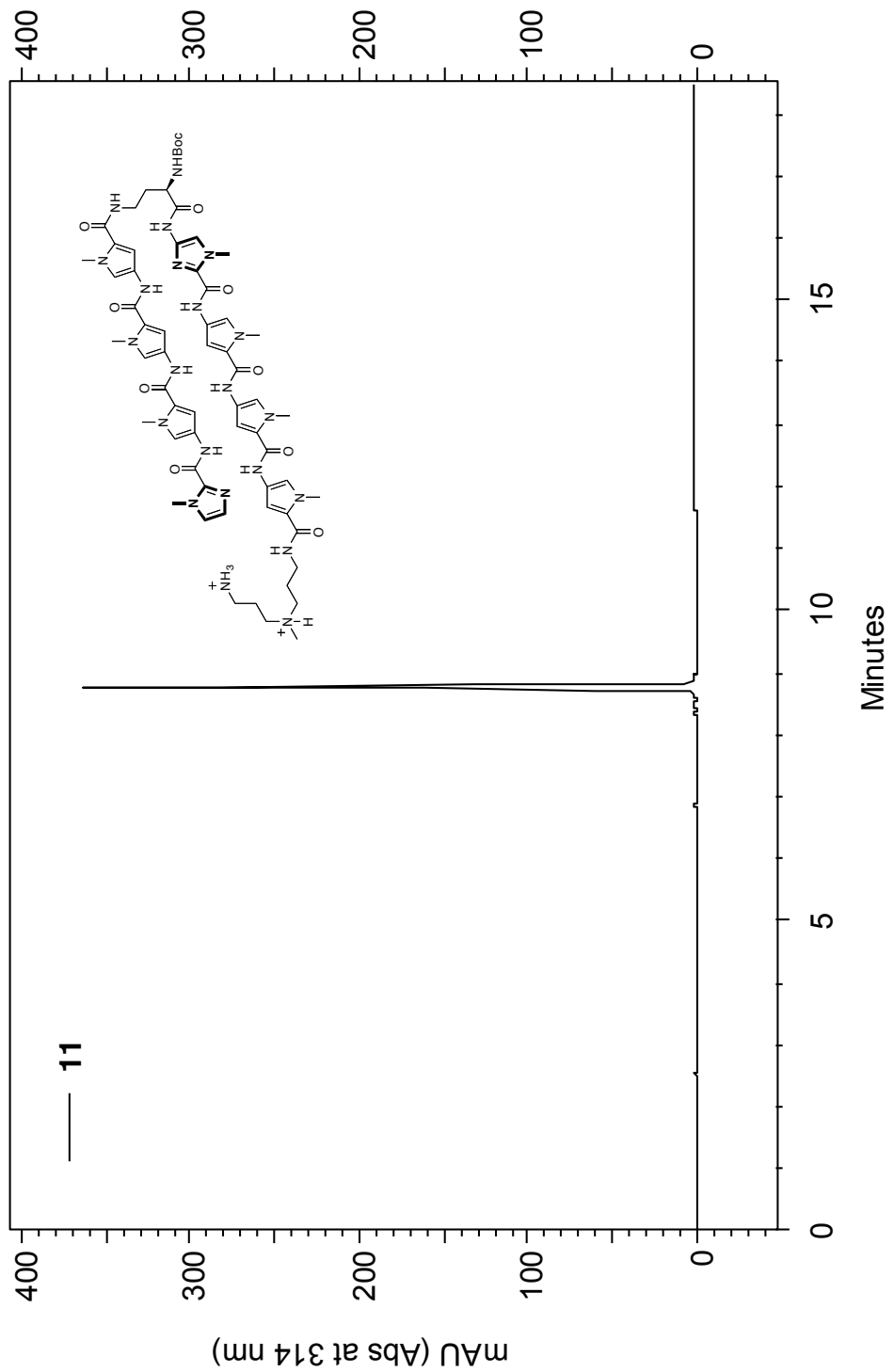
**12** ($C_{55}H_{68}N_{21}O_{9}^{+}$): expected $[M+H]^{+}$: 1166.6, observed $[M+H]^{+}$: 1166.5

**12-*(R,R)*** ($C_{65}H_{75}F_{3}N_{21}O_{11}^{+}$): expected $[M+H]^{+}$: 1382.6, observed $[M+H]^{+}$: 1382.7

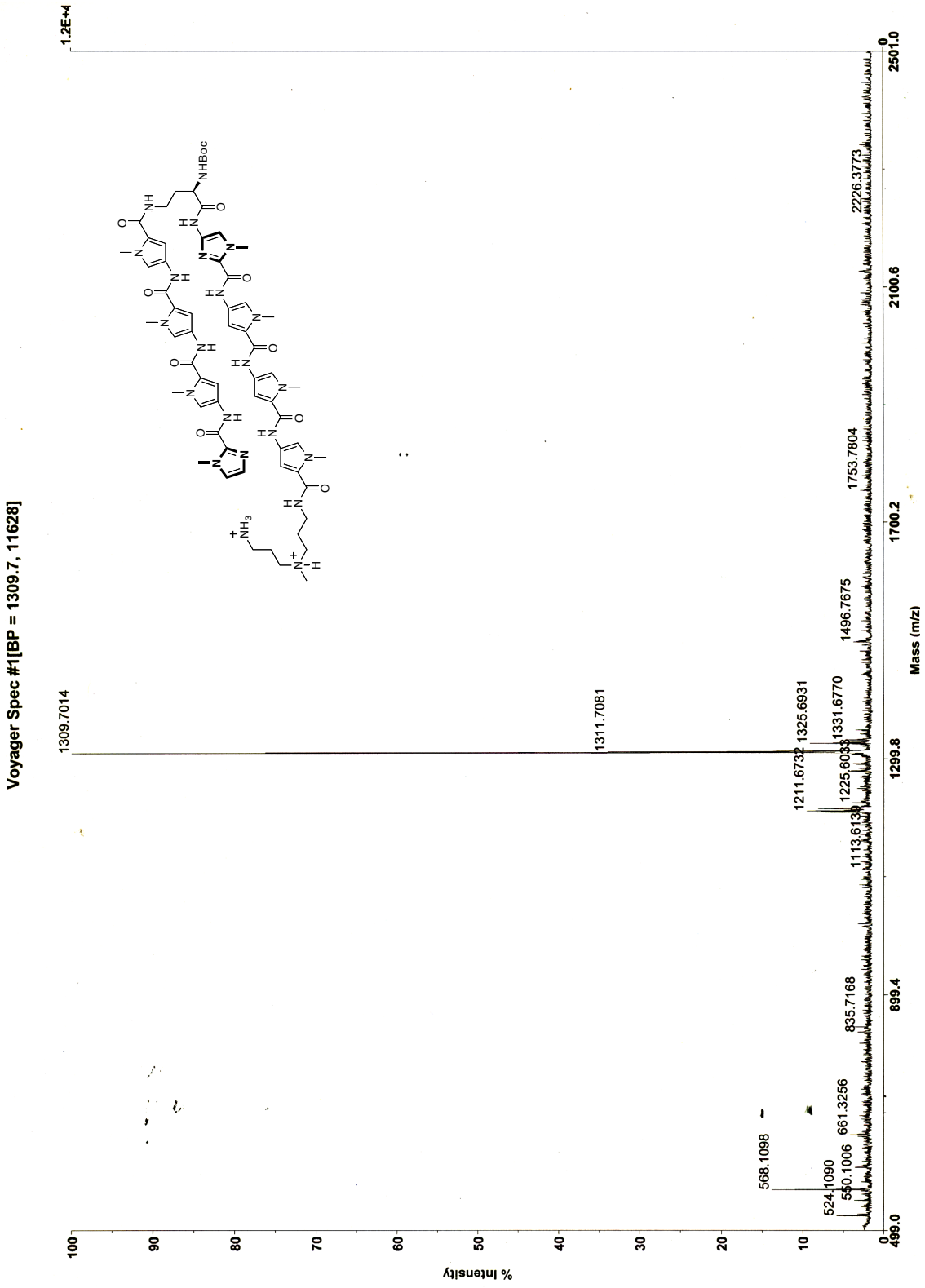
**12-*(R,S)*** ($C_{65}H_{75}F_{3}N_{21}O_{11}^{+}$): expected $[M+H]^{+}$: 1382.6, observed $[M+H]^{+}$: 1382.6

**20** ($C_{55}H_{68}N_{21}O_{9}^{+}$): expected $[M+H]^{+}$: 1166.6, observed $[M+H]^{+}$: 1166.5

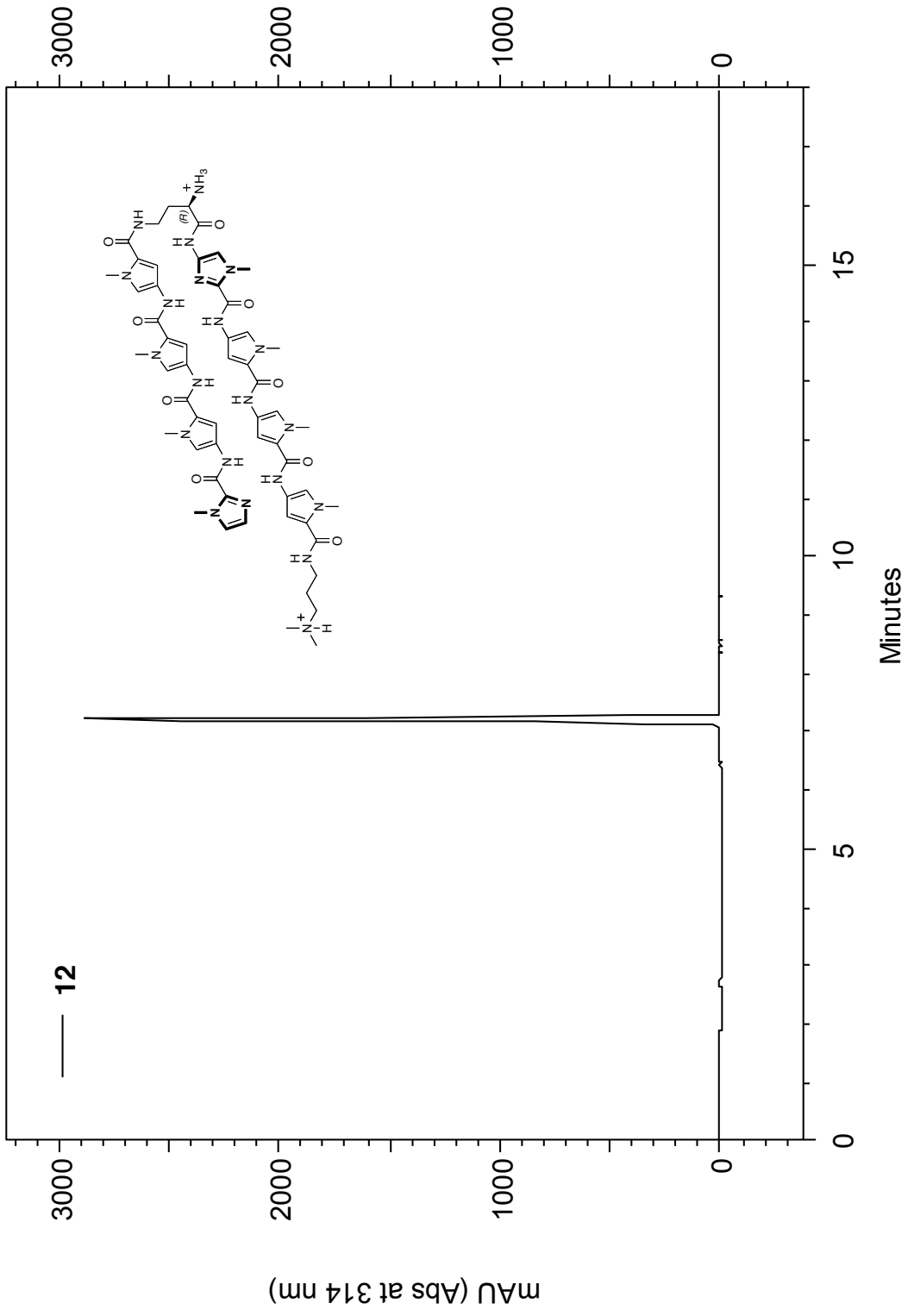
**20-*(R,R)*** ($C_{65}H_{75}F_{3}N_{21}O_{11}^{+}$): expected $[M+H]^{+}$: 1382.6, observed $[M+H]^{+}$: 1382.7



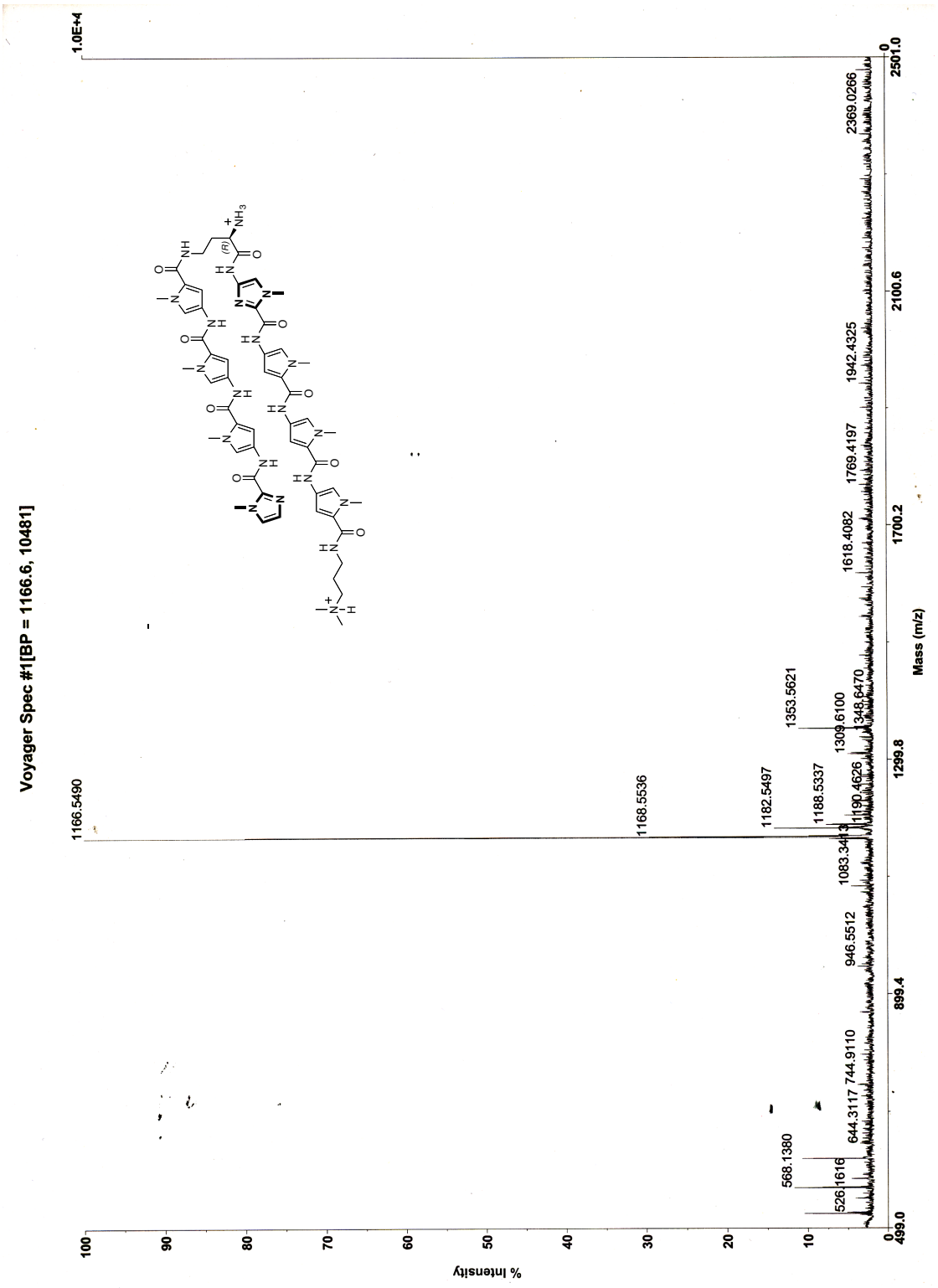

Analytical HPLC UV/Vis trace, compound **11**, 314 nm.



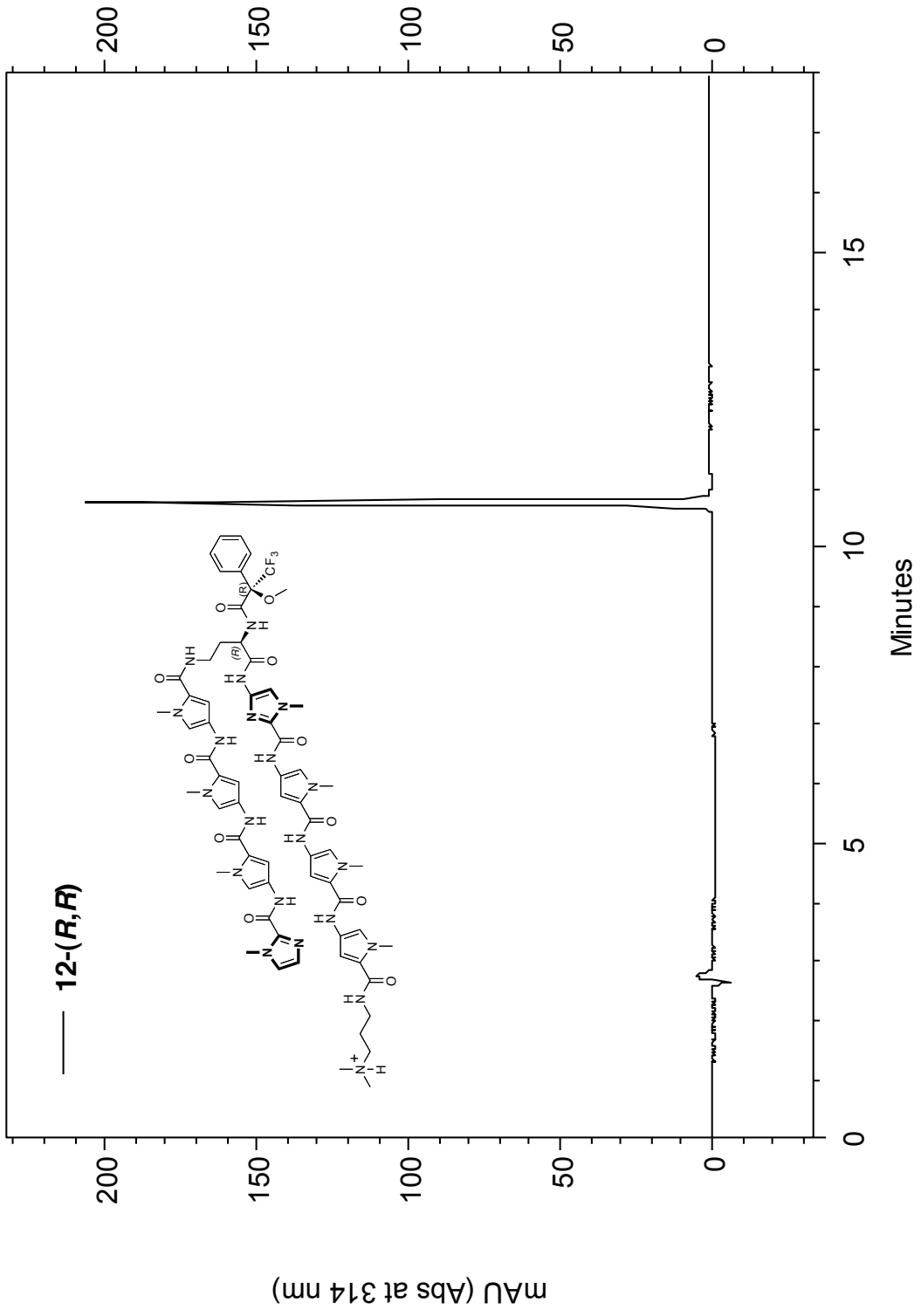

MALDI-TOF spectrum, compound **11**.



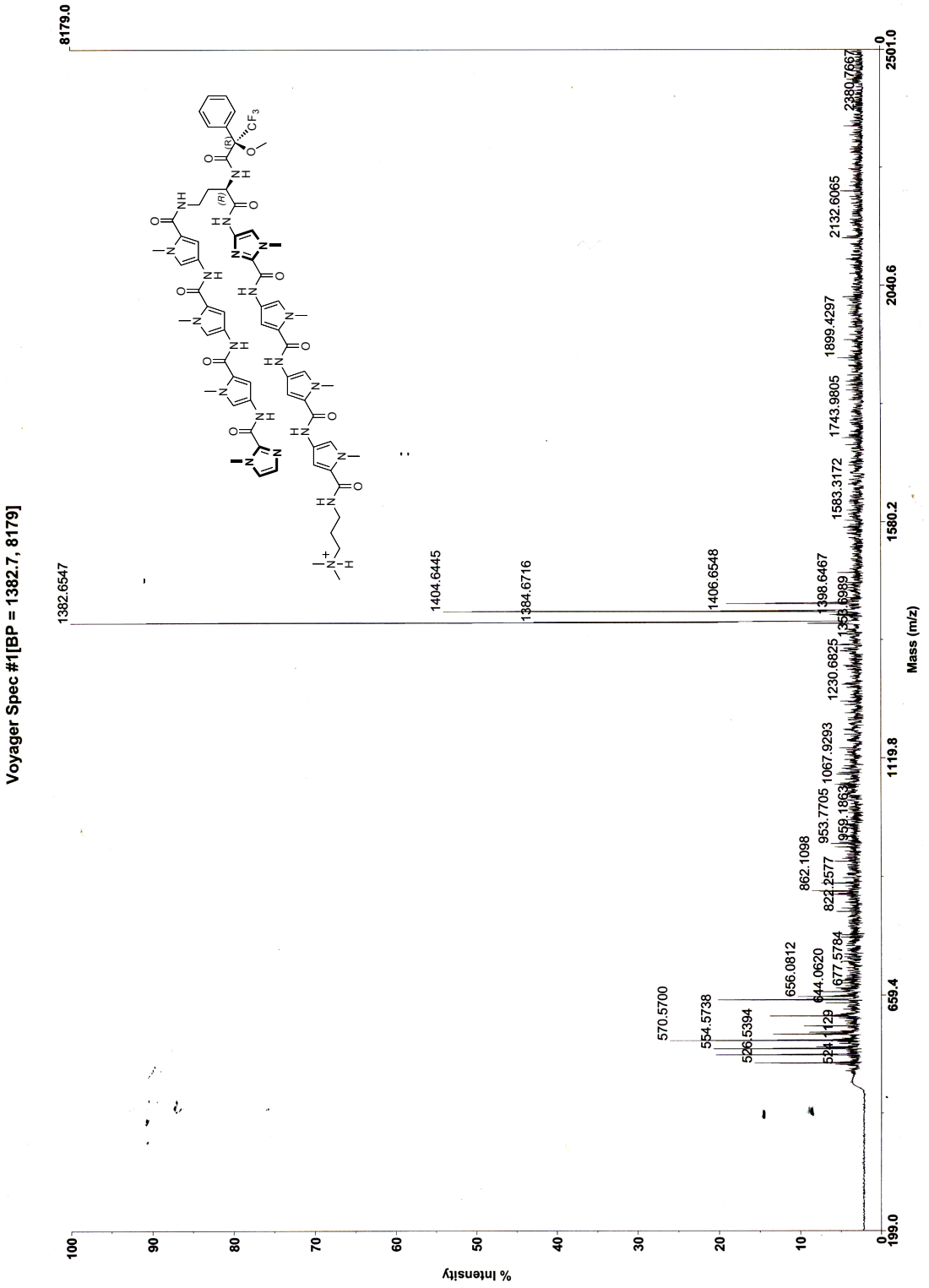

Analytical HPLC UV/Vis trace, compound **12**, 314 nm.



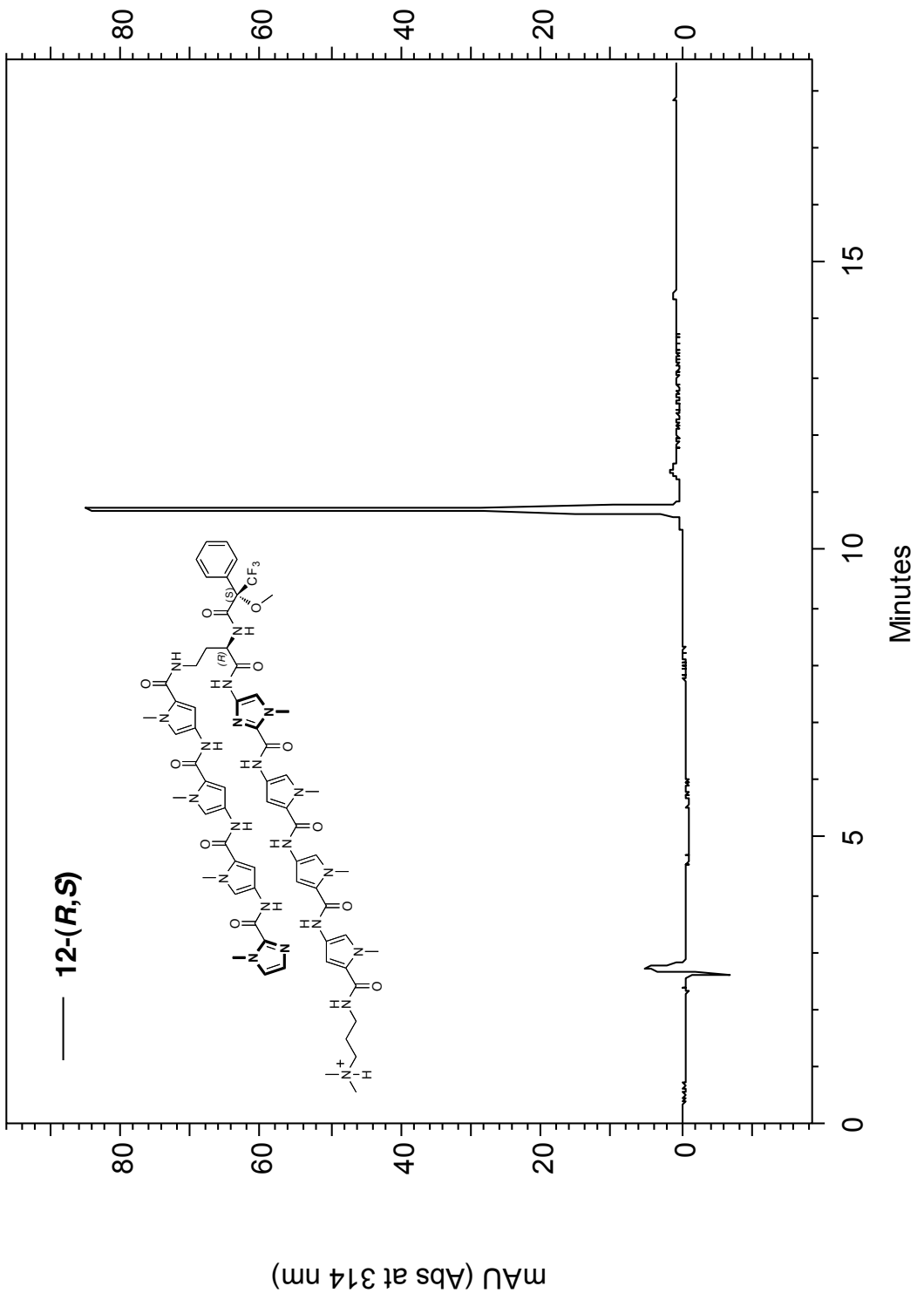

MALDI-TOF spectrum, compound **12**.



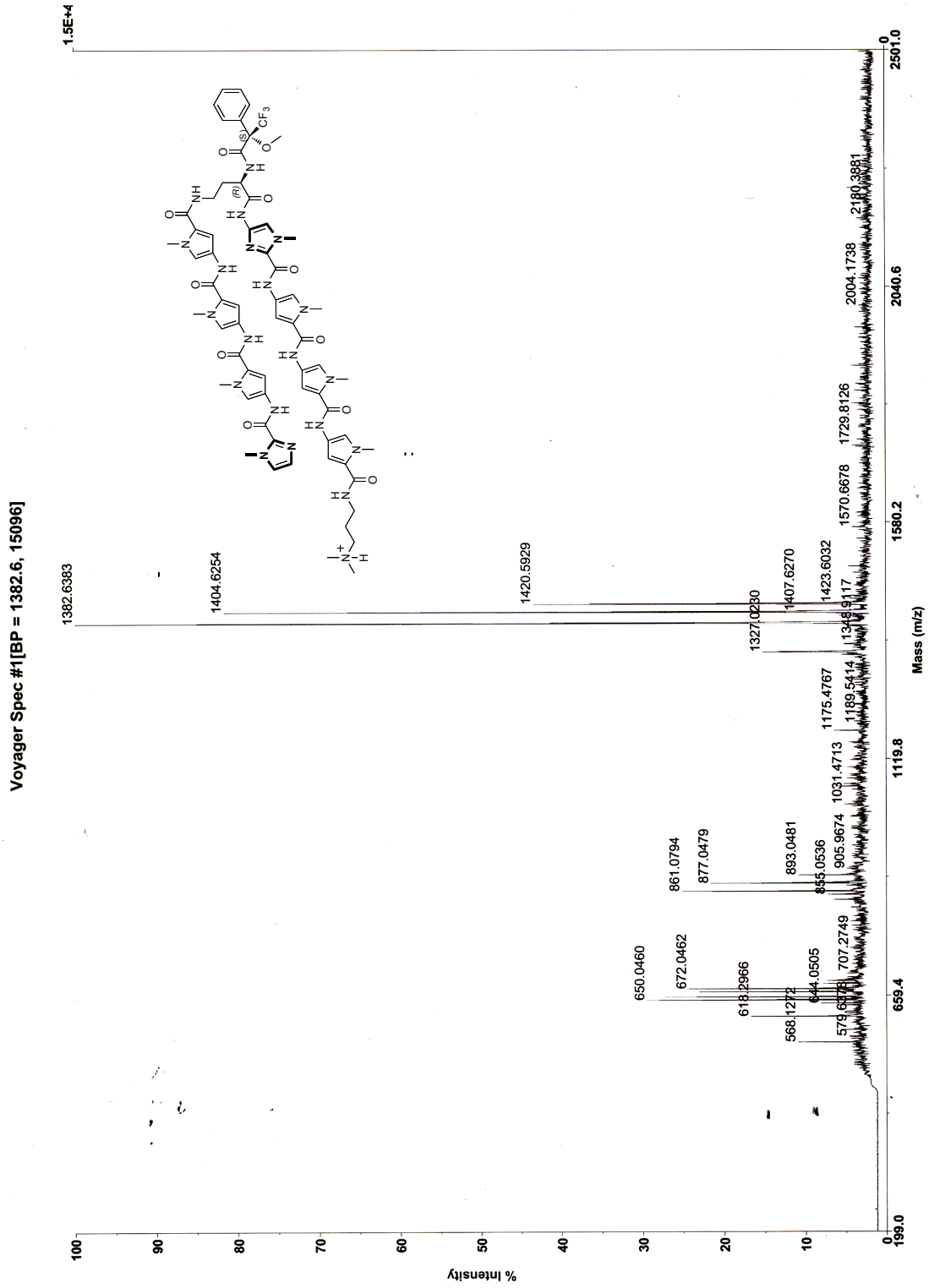

Analytical HPLC UV/Vis trace, compound **12-(*R*,*R*)**, 314 nm.



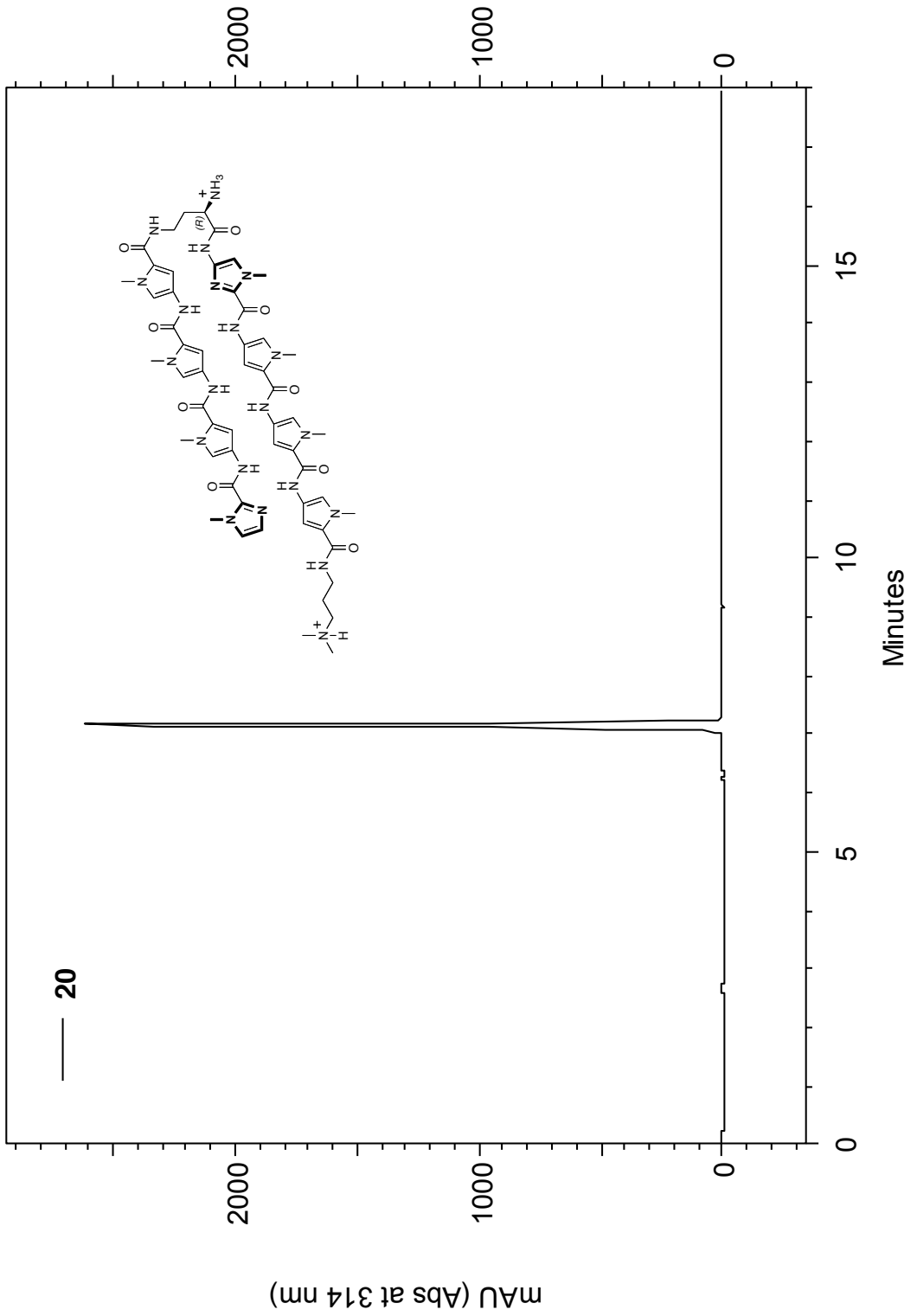

MALDI-TOF spectrum, compound **12-(*R*,*R*)**.



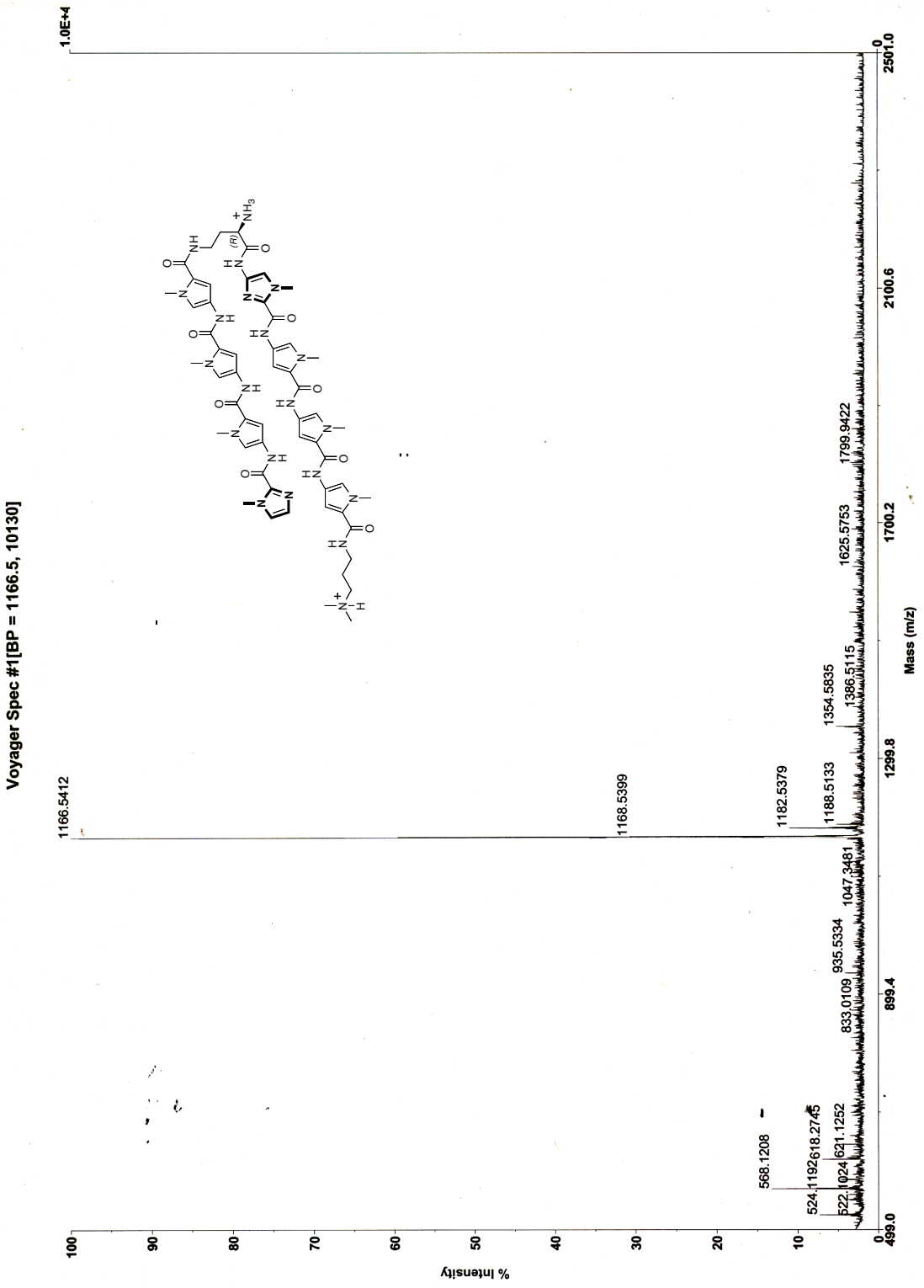

Analytical HPLC UV/Vis trace, compound **12-(*R*,*S*)**, 314 nm.



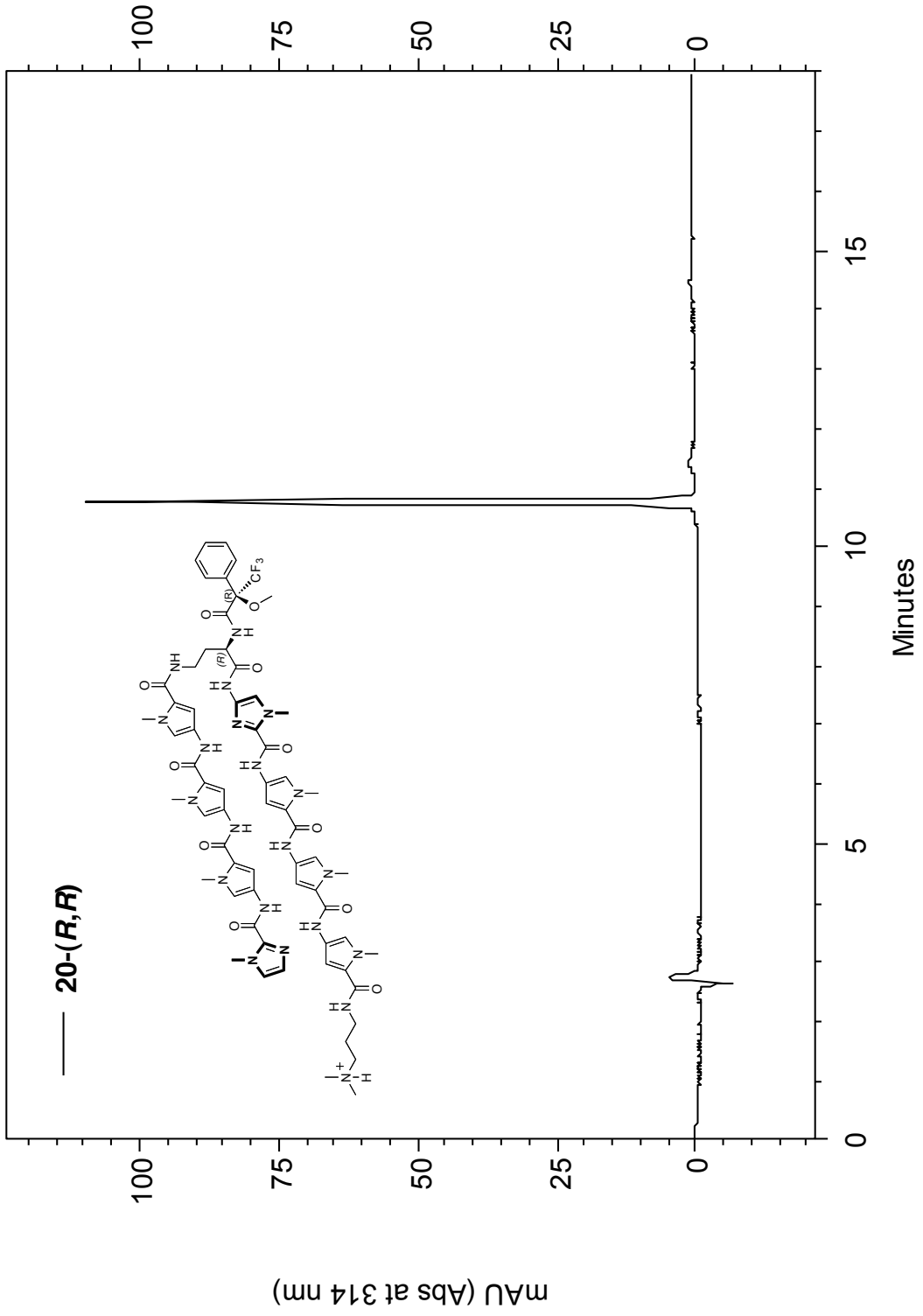

MALDI-TOF spectrum, compound **12-(*R*,*S*)**.



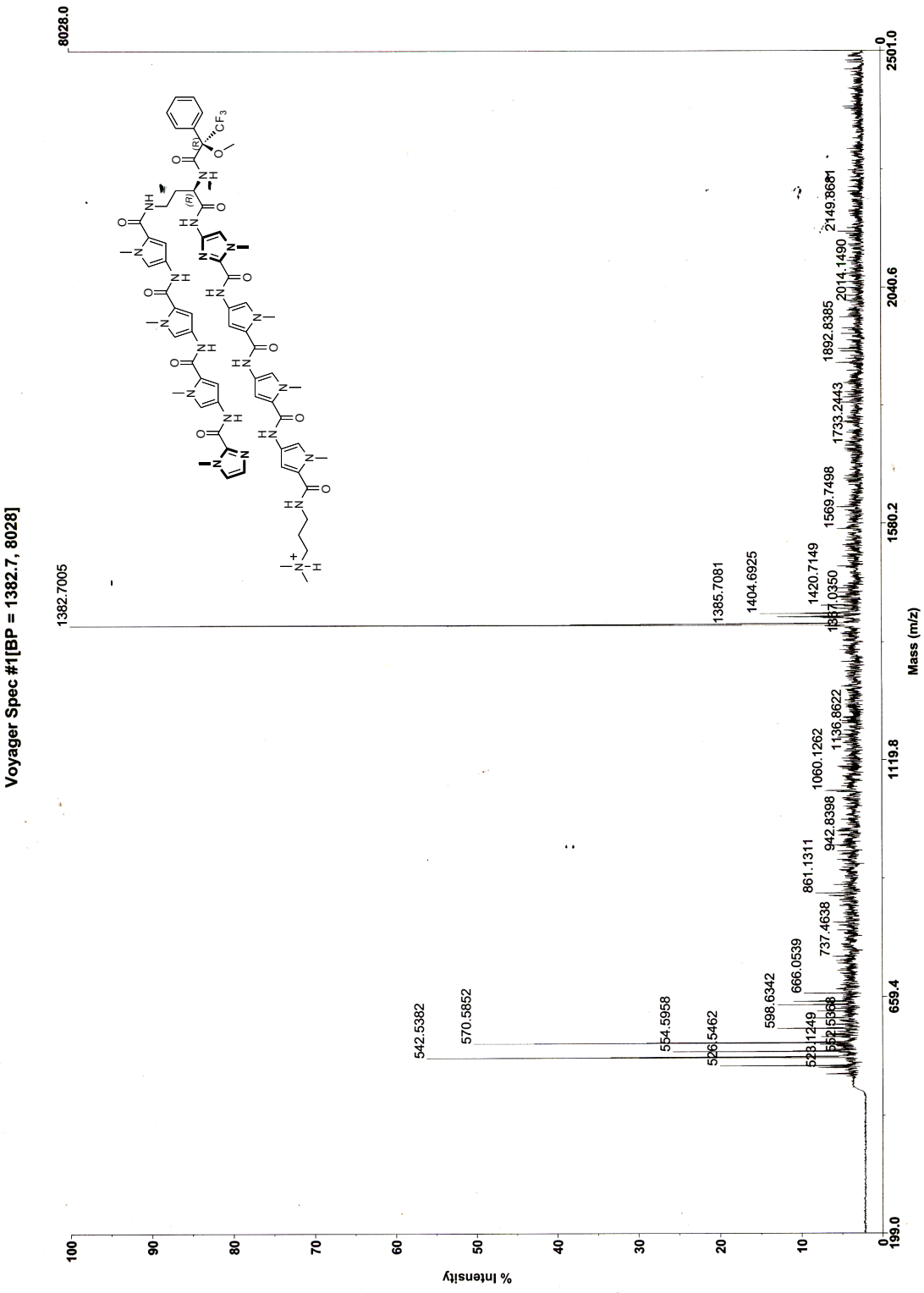

Analytical HPLC UV/Vis trace, compound **20**, 314 nm.



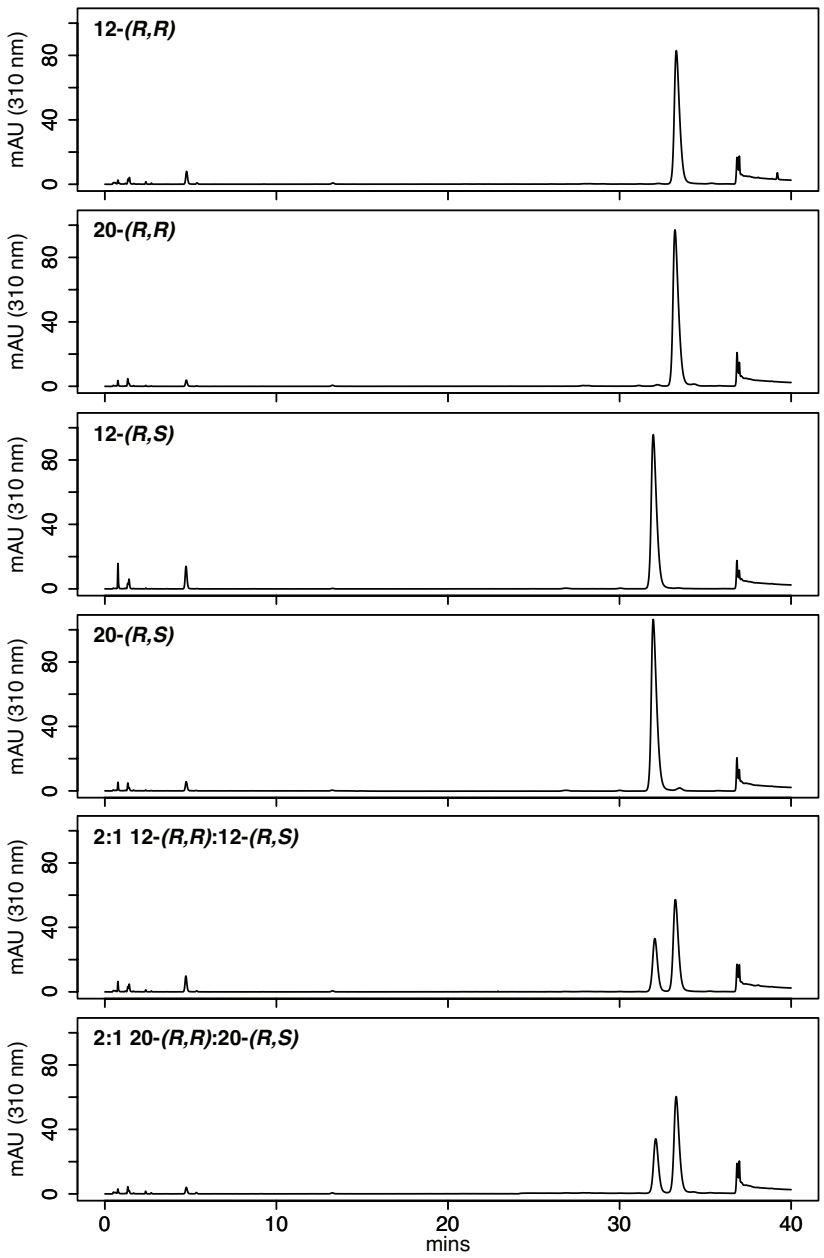

MALDI-TOF spectrum, compound **20**.




Analytical HPLC UV/Vis trace, compound **20-(*R*,*R*)**, 314 nm.




MALDI-TOF spectrum, compound **20-(*R*,*R*)**.




Analytical UPLC traces from Mosher amide derivatization experiment.

## *References*


1. Belitsky, J.M.; Nguyen, D.H.; Wurtz, N.R.; Dervan, P.B. *Bioorg. Med. Chem.* **2002**, *10*, 2767–2774.
2. (a) Baird, E.E.; Dervan, P.B. *J. Am. Chem. Soc.* **1996**, *118*, 6141–6146. (b) Corral, C.; Lasso, A.; Lissavetzky, J.; Sanchez Alvarez-Insua, A.; Valdeolmillos, A. M. *Heterocycles* **1985**, *23*, 1431–1435.
3. (a) Marques, M. A. Ph. D. Dissertation, California Institute of Technology, Pasadena, CA 2005. (b) Su, W.; Gray, S. J.; Dondi, R.; Burley, G. A. *Org. Lett.* **2009**, *11*, 3910–3913.
4. Nickols, N.G.; Dervan, P.B. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10418–10423.