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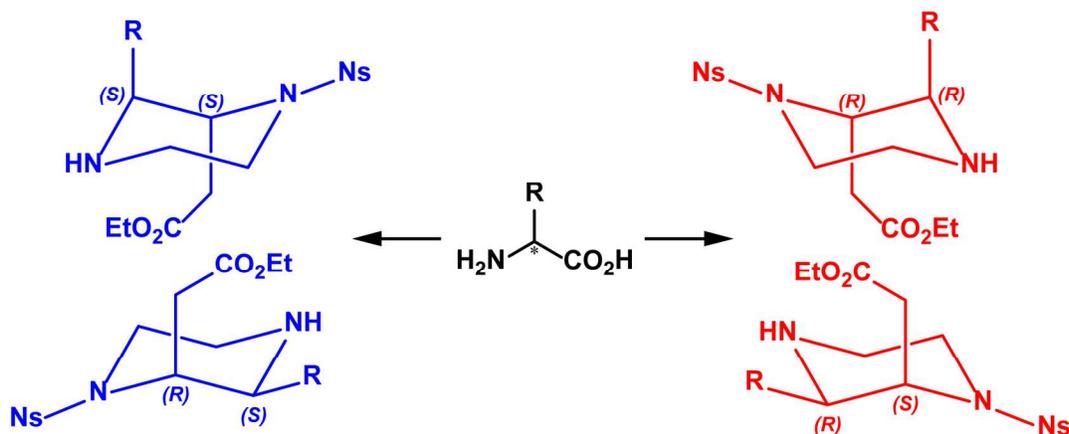
Synthesis of Enantiomerically Pure 3-Substituted-Piperazine-2-Acetic Acid Esters as Intermediates for Library Production

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ABSTRACT: The piperazine heterocycle is broadly exploited in FDA-approved drugs and biologically active compounds, but its chemical diversity is usually limited to ring nitrogen substitutions, leaving the four carbon atoms underutilized. Using an efficient six step synthesis, chiral amino acids were transformed into 3-substituted-piperazine-2-acetic acid esters as diastereomeric mixtures whose *cis* and *trans* products (dr: 0.56→2.2:1 respectively) could be chromatographically separated. From five amino acids (both antipodes), a complete matrix of 20 mono-protected chiral 2,3-disubstituted piperazines was obtained, each as a single absolute stereoisomer, all but one in multi-gram quantities. In keeping with our overall purpose of constructing 3-dimensional fragment libraries, these diverse and versatile piperazines can be functionalized on either nitrogen atom, allowing them to be used as scaffolds for parallel library synthesis or intermediates for the production of novel piperazine compounds.

Introduction

Small-molecule screening underlies the earliest phase of the chemical probe and therapeutic discovery process.¹ A convincing body of data supports the premise that small-molecule libraries built around the principle of chemical diversity will lead to a higher hit rate across wider swaths of biological target space.²⁻⁴ Accordingly, the effort to populate small-molecule collections with structurally diverse molecular entities in screening collections stands as a major intellectual and strategic goal for synthetic organic chemistry.

Our efforts to generate small-molecule libraries optimized for biological discovery have focused on the synthesis of scaffold families in which systematic variation of structural features on the core scaffold generates a complete matrix of constitutional isomers (Figure 1). Within the hierarchy of a scaffold family, systematic chemical diversity can be applied to establish a core scaffold having different substitution patterns. Each core scaffold offers unique regiochemical substitution possibilities, and if the substituents are placed on sp^3 -carbon atoms, then each regioisomer affords both relative and absolute stereochemical configurations. The complete set of regio and stereoisomers can be elaborated further for library synthesis. Systematic chemical diversity provides an effective means of sampling chemical space by exploiting different vector substitutions that can in principle lead to greater molecular recognition potential toward a variety of biological targets. The generation of optimal screening libraries based on these principles is a major objective of our laboratory.

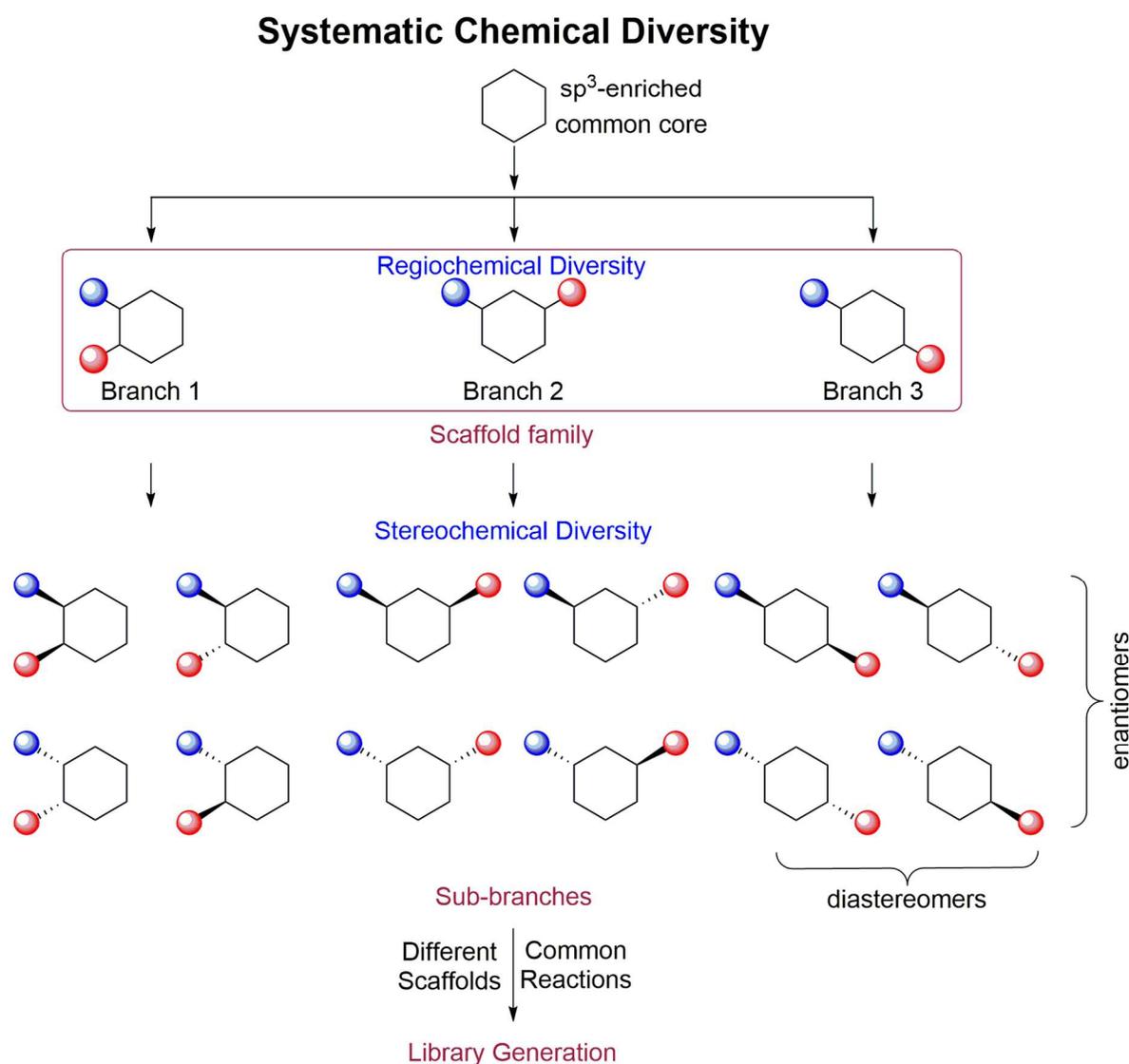


Figure 1. A general representation of a scaffold family and the concept of systematic chemical diversity.

The piperazine heterocycle is frequently encountered within FDA approved drugs and other reported biologically active compounds;⁵⁻⁹ however, its derivatization has mostly been limited to 1,4-substitution on the nitrogen atoms. Piperazines have features that can be exploited for rendering structural diversity including, two sites of nitrogen diversification and four sp^3 -carbon atoms at which stereochemical diversity can be generated. We perceived the four piperazine carbon atoms as opportunities to apply systematic chemical diversity principles and produce a three-branch family of functionalized enantiomerically pure piperazines from commercially available chiral starting materials. The three branches of the scaffold family are demarcated by their substitution patterns as 2,3, 2,5 and 2,6 and the familial relationship is illustrated in Figure 2.

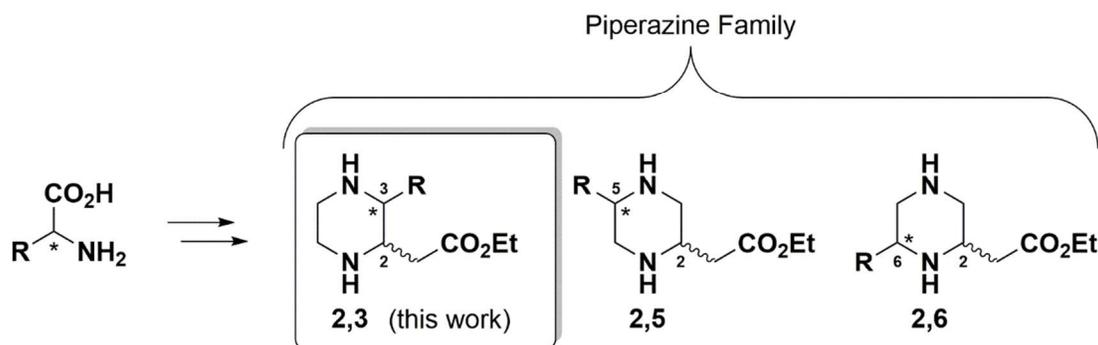


Figure 2. Scaffold family of disubstituted piperazine-2-acetic acid ester regioisomers.

The synthesis of the 2,6-piperazine acetic acid ester branch has been previously reported by us¹⁰ and in this report we describe our efforts toward the preparation of the 2,3-branch of the scaffold family.

Results and Discussion

Our targeted scaffold family represents a significant departure from how piperazines are normally employed in that the carbon atoms are used to add functionality. Our interest in deploying these scaffolds for parallel synthesis of screening libraries required us to develop syntheses that could be scaled to meet our material requirements. The acetate ester side chain was included to both enable the synthesis of the piperazine ring and to insure, after hydrolysis, that the derived diversified compounds would have enhanced solubility in aqueous media while minimizing the potential for aggregation even at high concentrations (mM range). In certain cases the carboxylic acid could also serve as a site for further diversification.

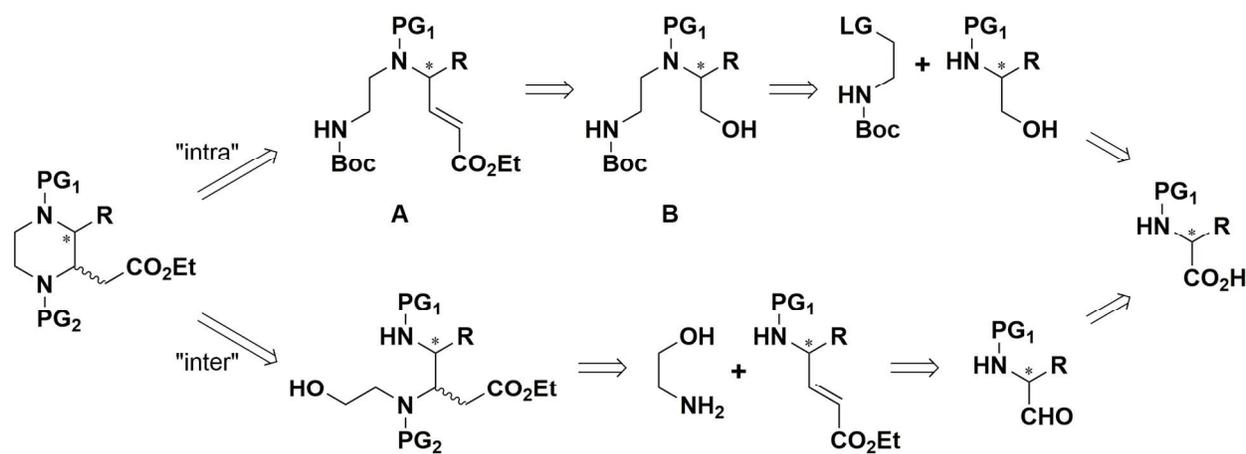
Piperazines that are 2,3-disubstituted can be formally considered to be products arising from the bridging of the nitrogens of 1,2-ethylenediamines with an ethylene linker.¹¹ There is precedent for the conversion of certain 1,2-ethylenediamines to 2,3-disubstituted piperazines utilizing various sources for the two-carbon bridge. The simplest example, ethylene glycol, has been reported to participate in the direct, one step conversion of 1,2-diamines to 2,3-disubstituted

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3 piperazines under Pd/MgO,¹² iridium¹³⁻¹⁶ or ruthenium¹⁷ catalysis. Alternatively, 2-bromoethyl
4 diphenylsulfonium triflates are reported to effect conversion of 1,2-diamines to 2,3-disubstituted
5 piperazines through a double *N*-alkylation mechanism.^{16,18,19} Related methodology employs
6 vinyl diphenylsulfonium triflates, obtained by treatment of the aforementioned 2-bromoethyl
7 diphenylsulfonium triflates with base, followed by reaction with 1,2-diamines through a tandem
8 aza-Michael addition followed by intramolecular *N* alkylation.^{18,20} Another report describes a
9 tandem aza-Michael reaction of 1,2-diamines to phenyl vinyl selenone followed by
10 intramolecular *N* alkylation.²¹ Two step protocols have also been described using chloroacetyl
11 chloride²²⁻²⁴ or α -dicarbonyl compounds as the source of the two carbon bridge. The
12 employment of oxalate esters²⁵⁻²⁹ and ethyl glyoxylate³⁰ to convert 1,2-diamines to 1,4-
13 piperazine-diones is followed by reduction of the cyclic bis-amides to give 2,3-disubstituted
14 piperazines. Each of the aforementioned methodologies is characterized by an initial
15 stereoselective synthesis of a chiral 1,2-diamine that requires controlling the establishment of
16 two vicinal stereogenic centers. From the perspective of generating scaffold families, this is
17 disadvantageous given that individual syntheses would be required for each 1,2-diamine
18 stereoisomer. We were motivated by our previous efforts involving a route toward producing a
19 mixture of enantiomerically pure 2,6- disubstituted piperazines diastereomers in a single pot.
20 The strategy relied on a relatively non-selective generation of diastereomeric piperazines
21 followed by an efficient and scalable chromatographic separation of the diastereomeric
22 products. We surmised that a similar design for establishing the requisite 2,3-piperazine scaffold
23 branch could be achieved using starting materials from the chiral pool and the development a
24 synthesis for the non-selective formation of a second, vicinal stereocenter to generate a mixture
25 of enantiomerically pure 2,3-piperazine diastereomers. Accordingly, use of the opposite
26 enantiomer of the starting material from the chiral pool could produce the antipodes of all the
27 scaffolds.
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35 Retrosynthetic analysis of the 2,3-substituted members of the piperazine-2-acetic acid ester
36 scaffold branch is depicted in Scheme 1. The paths labeled “inter” and “intra” refer to the
37 manner in which the second stereocenter is formed. The “intra” path envisions the formation of
38 the second stereocenter by an intramolecular aza-Michael reaction of *N*₁ to an acrylate moiety,
39 analogous to the method used to prepare the previously reported 2,6-substituted piperazine
40 family branch. The acrylate is, in turn, obtained from an amino alcohol that is derived from an
41 amino acid. We anticipated that the intramolecular aza-Michael reaction would be moderately
42 diastereoselective in a manner observed for the 2,6 case (*dr* \approx 3:1). The “inter” path envisions
43 the formation of the second stereocenter by an intermolecular aza-Michael reaction of
44 ethanolamine to an acrylate also derived from the same amino acid. In the “inter” path option we
45 anticipated that the diastereoselectivity of the aza-Michael reaction would be marginal at best,
46 which for our purposes is the better outcome since we seek to acquire large quantities of both
47 diastereomers that emerge at the end of the synthesis. Following an appropriate *N*-protection/*O*-
48 activation sequence, the amino acid derived nitrogen would be unmasked and ring closure
49 would occur via intramolecular S_N2 displacement.
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54 There is limited precedent for both pathways *en route* to 2,3-substituted piperazines. For the
55 “intra” route the unmasking a Boc protected amine under acidic conditions followed by
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neutralization to effect an intramolecular aza-Michael cyclization has been reported.³¹⁻³⁷ Other examples have been demonstrated utilizing the trityl group on the incipient nucleophilic nitrogen, unmasked under acidic conditions³⁸⁻⁴⁰ or directly employed under basic conditions⁴¹. The removal of an Fmoc protecting group prior to an aza-Michael addition has been reported^{35,42} as well as the aza-Michael of an amide nitrogen to an acrylate pre-activated with TBSOTf⁴³. There are also examples of direct acid catalysis without preactivation when the attacking nucleophile is an aniline nitrogen⁴⁴ as well as base promoted aza-Michael of Cbz protected nitrogen^{45,46,47}. For the "inter" route there are limited examples of ethanolamine, either directly or as a synthon, being used as an aza-Michael donor.⁴⁸⁻⁵⁰ Closure of a piperazine ring from β -hydroxyamine precursors has generally been carried out by either halogenation,⁵¹ mesylate displacement^{52,53} or with the Mitsunobu protocol⁵⁴⁻⁵⁶. None of these precedents had been applied toward the production of complete stereoisomeric matrices of 2,3-piperazine products.



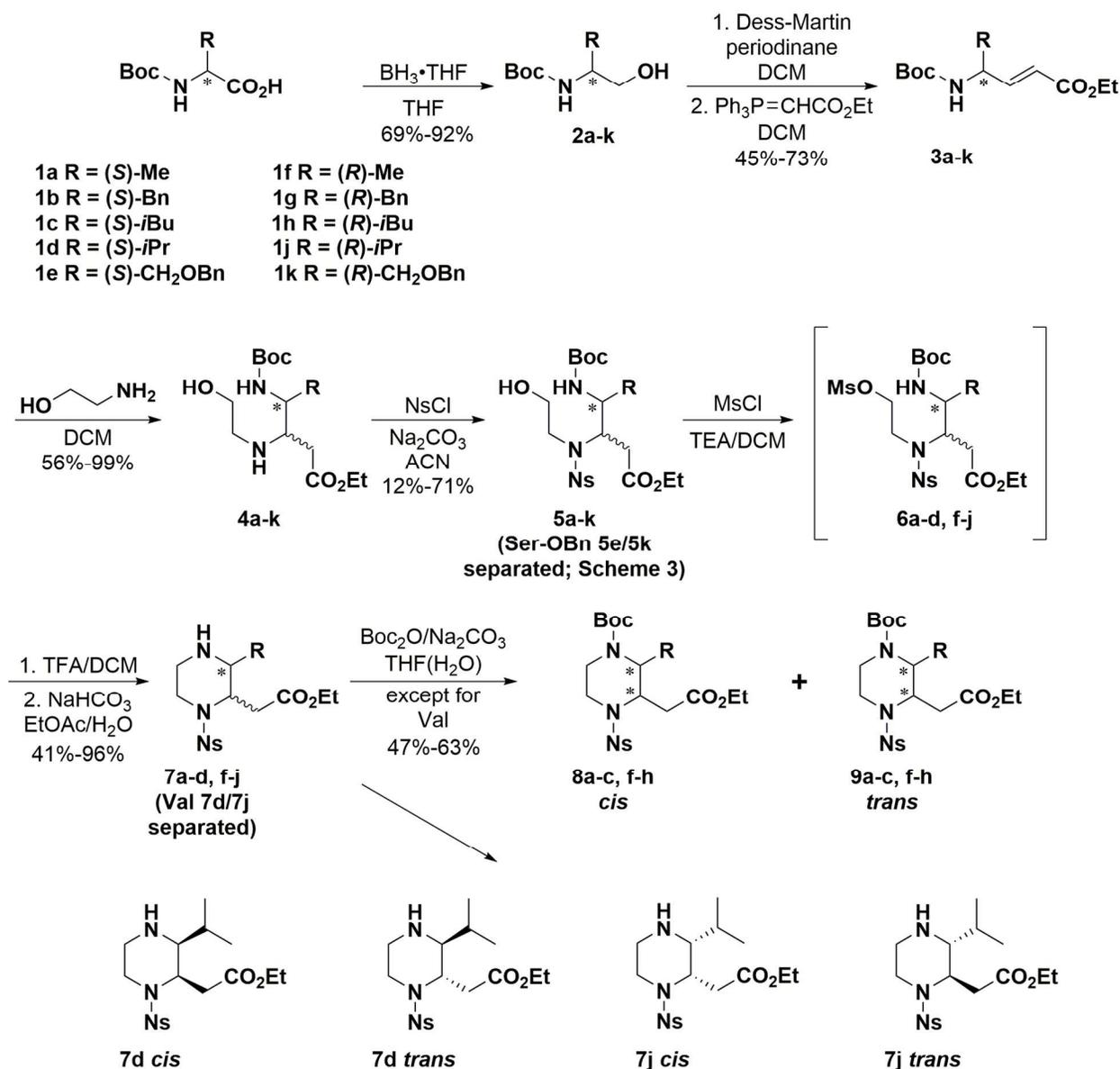
Scheme 1. Retrosynthetic analysis of 3-substituted-piperazine-2-acetic acid ethyl esters.

For simplicity of design, systematic chemical diversity would ideally utilize a common bond forming strategy for each of the various branches of the scaffold family. In this manner, structurally different building blocks could be carried through the same bond forming maneuvers to generate different scaffold family members. Thus, in keeping with the successful precedent established during the 2,6 scaffold work, initial studies focused on the "intra" pathway. We quickly encountered two major, ultimately insurmountable problems with the "intra" approach. The first was the preparation of acrylate **A** from alcohol **B**, a two-step process that involved the oxidation of **B** to the intermediate aldehyde (not shown) followed by exposure of the aldehyde to (carbethoxymethylene)triphenylphosphorane. This conversion was uniformly unsatisfactory, consistently affording troublesome mixtures containing varying amounts of the desired acrylate **A** in poor yields. Even more troublesome was the surprising failure of the intramolecular aza-Michael addition to afford the piperazine scaffold. Similar to natural product total synthesis, systematic chemical diversity rests on goal of generating a predetermined final set of structures and is agnostic to the route taken. Therefore, a unique synthesis pathway may need to be employed toward the generation of complete scaffold families. Thus we adopted the "inter" retrosynthetic strategy (Scheme 2) which offered us the advantages of scalability and access to

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3 equal amounts of both diastereomers. The *N*-Boc amino acids **1a-k** were reduced to their
4 corresponding alcohols **2a-k** by exposure to $\text{BH}_3 \cdot \text{THF}$. Subsequent oxidation using Dess-Martin
5 conditions generated the corresponding aldehydes (not shown). The crude aldehydes were
6 immediately treated with (carbethoxymethylene)triphenylphosphorane to afford the acrylates **3a-**
7 **k**, all having *trans* geometry.
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10 The potential for racemization of the intermediate aldehydes prepared using the above
11 methodology was a major concern. For that reason, we undertook the synthesis of racemic **2a**,
12 as an exemplar, using the same method as shown in Scheme 2. Both the chiral and the racemic
13 alcohols were converted to their respective *trans* acrylates **3a** as described. Chiral HPLC
14 analysis of both materials showed no loss of enantiomeric purity in the chiral acrylate **3a**.
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17 Acrylates **3a-k** were reacted with ethanolamine (4eq). As anticipated, the aza-Michael addition
18 occurred at rt with modest diastereoselectivity, affording mixtures of diastereomers **4a-k**. The
19 relative stereochemistry of the Michael adducts was deduced by a combination of NMR
20 spectroscopy and X-ray crystallography from the derived piperazines later in the sequence as
21 described below (see Table 1). Treatment of **4a-k** with $\text{NsCl}/\text{Na}_2\text{CO}_3$ gave the *N*-nosylated
22 products **5a-k** which were purified by chromatography.
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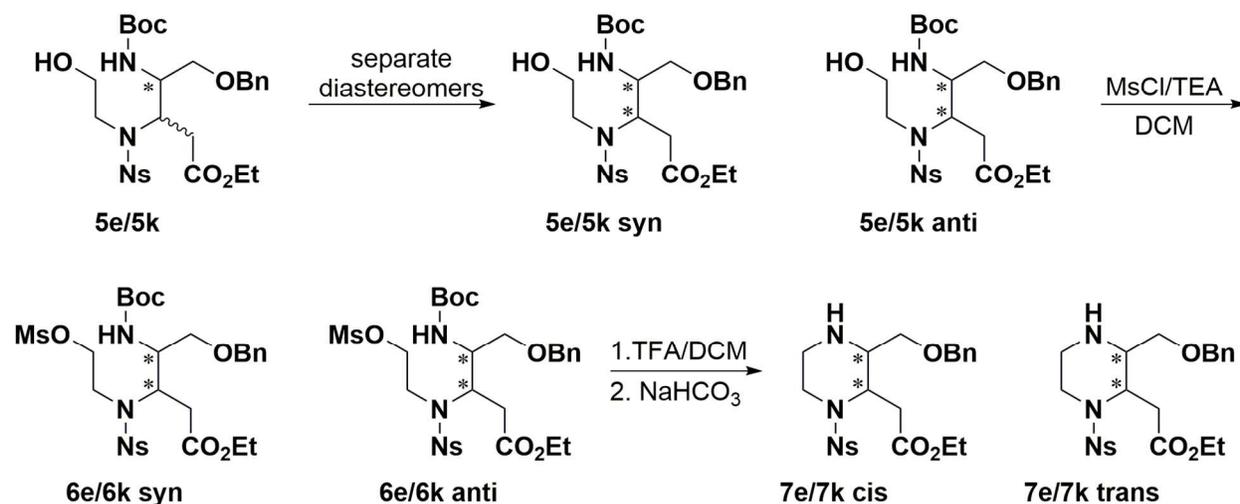
Scheme 2. Synthesis of 3-substituted-piperazine-2-acetic acid ethyl esters.

At this point the serine-*O*-benzyl ether derived intermediates **5e** and **5k** were set aside for chromatographic separation of diastereomers as described below (Scheme 3). The remaining intermediates **5a-d** and **5f-j** were advanced through the sequence shown in Scheme 2. Reaction of diastereomers **5a-d** and **5f-j** with MsCl/TEA gave the *O*-mesylated intermediates **6a-d** and **6f-j** which were carried forward in crude form. Treatment of **6a-d** and **6f-j** with TFA resulted in the unmasking of the amino acid derived nitrogen. Neutralization afforded the piperazines **7a-d** and **7f-j**. Piperazines **7d**/**7j** could be separated into their individual diastereomers **7d cis/trans** and **7j cis/trans**. The separation of piperazines **7a-c** and **7f-h** proved to be difficult but Boc reprotection of the nitrogen at the 4-position rendered the remaining diastereomers **8a-c cis**, **9a-c trans**, **8f-h cis** and **9f-h trans** separable by normal

phase column chromatography. The strategic preparation and separation of diastereomeric mixtures is key to the efficient implementation of the systematic chemical diversity strategy.

There were some notable variations in the Scheme 2 sequence. In the case of valine, which is the only amino acid with branching at the β carbon that was successfully employed in this work, the rate and yield of the Michael addition of ethanolamine to the acrylates **3d/3j** were significantly reduced. In all other cases, such as the phenylalanine derived acrylates **3b/3g** where there is no branching at the β carbon, the reaction went to 100% completion in 48h at rt to afford the desired Michael adducts as the major product. In the case of valine, the reaction required a week to approach completion (>90% by LCMS). Heating the reaction only led to complex mixtures. Even when carried out at rt, the conjugate addition to acrylates **3d/3j** gave a mixture of products in which adducts **4d/4j** were present in significant but varying amounts. After advancing the Michael adducts **4d/4j**, as described above, to their derived piperazines **7d/7j** each pair of diastereomers was separated into its individual stereoisomers **7d cis**, **7d trans**, **7k cis** and **7k trans** as described above.

In the case of the serine-*O*-benzyl ether derived intermediates **5e/5k**, diastereomers **5e syn/anti** and **5k syn/anti** could be individually isolated prior to cyclization (Scheme 3). The four intermediates were advanced through their derived mesylates **6e syn/anti** and **6k syn/anti** to the piperazines **7e cis**, **7e trans**, **7k cis** and **7k trans**.



Scheme 3. Synthesis of piperazine scaffolds derived from serine-*O*-benzyl ether.

Each cyclization reaction gives two products of the target molecular mass that are resolved by silica gel chromatography. Analytical chiral HPLC shows that each product is enantiomerically pure, proving that the amino acid derived chiral center has not racemized *en route* to the final product. NMR spectra and assignments using ^{13}C and ^{15}N HMBC confirm the compound identities, establishing that the two products are the expected diastereomers. We use ^{13}C NMR chemical shifts of the C_5 and C_6 carbons to identify the 2,3 *cis* and 2,3 *trans* products; these identifications are confirmed with other NMR observables. From the relative stereochemistry of

the products and the chirality of the starting material, we infer the absolute stereochemistry of the final piperazine products. The approach is presented for compounds **7d cis** and **7d trans** (dr = 1:1.1) as shown in Figure 3.

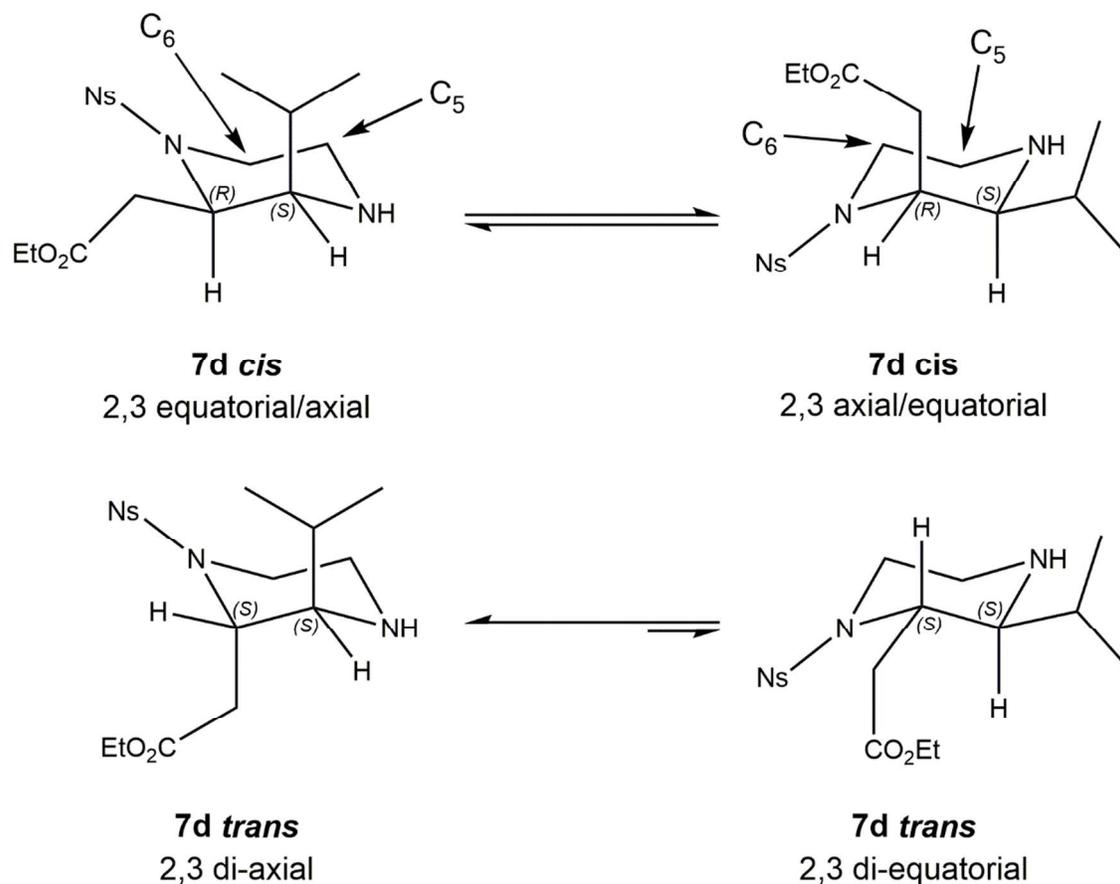


Figure 3. NMR analysis of conformational states of compounds **7d cis** and **7d trans**.

In *trans* products, substituents at C_2 and C_3 are di-axial to minimize allylic 1,3-strain with the nosyl amide as well as to avoid steric clashes that would occur between adjacent di-equatorial groups. Axial carbon substituents at C_2 and C_3 will induce 6ppm upfield perturbations of the ^{13}C chemical shifts of both the C_6 and C_5 atoms of the piperazine ring due to three-bond γ -gauche interactions.^{57,58} In *cis* products, which equilibrate between 2,3-axial/equatorial and 2,3-equatorial/axial states, only one of the remote atoms (C_5 or C_6) will experience the γ -gauche interaction at a given time, and rapid equilibration averages the shift perturbation over C_5 and C_6 . The sum of the C_5 and C_6 ^{13}C shifts in the *trans* product, which experiences two γ -gauche interactions with remote ring substituents, should be about 6 ppm less than for the *cis* product, which experiences only one.⁵⁹ For **7d** the sum of these chemical shifts in the minor product (C_5 : 46.99 ppm, C_6 : 41.56 ppm) is 7.38 ppm greater than in the major product (C_5 : 39.33 ppm, C_6 : 41.84 ppm), indicating that the major product is *trans*. The X-ray crystal structure of the major diastereomer starting from (*S*)-Val, obtained by crystallization from hexane/DCM, confirms that the major product is **7d trans** (Figure 4).

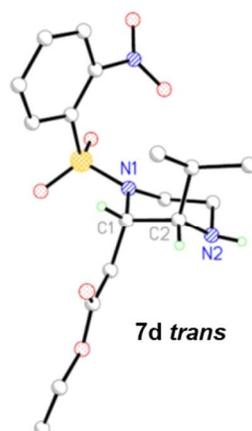
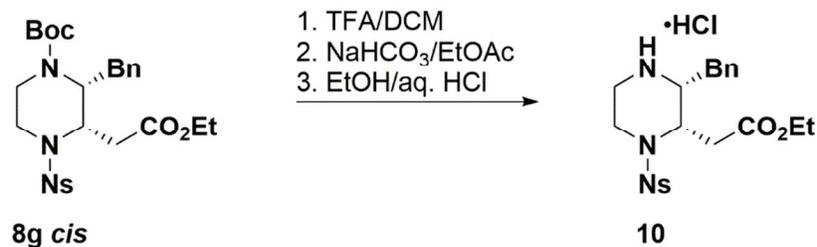


Figure 4. X-ray structure of **7d trans**

Solution NMR data that further confirms the identity of these diastereomers include ^{13}C linewidth increases at lower temperature for the *cis* but not the *trans* product, consistent with slow equilibration between *cis* ring conformers and the presence of only a single ring conformer for the *trans* product. For the *trans* product, NOEs between the C_2 substituent methylene protons and the H_6 axial proton, and between the C_3 substituent methine proton and the H_5 axial proton, confirm a *trans* di-axial configuration of the 2,3-substituents (for products with a Boc substituent at N_4 , the presence of Boc rotamers complicates but does not prevent this NOE analysis). Conformational averaging limits our ability to infer configuration of the *cis* product from NOEs or J couplings. All observed $^3J_{\text{HH}}$ and $^3J_{\text{CH}}$ for *trans* products are consistent with the assigned stereochemistry.

Stereochemical inferences about all other pairs of diastereomers have been similarly based on ^{13}C chemical shift differences at positions C_5 and C_6 , variable temperature NMR, NOEs, and 3J couplings. Crystal structures of both the major and minor products derived from (*S*)-Phe further confirm the validity of this approach. Because in our synthetic approach we make two diastereomers from a single chiral starting material, we can always compare informative ^1H chemical shifts, ^1H multiplet structure, ^{13}C chemical shifts, and ^1H - ^{13}C three-bond J couplings of both diastereomers as described above to confirm the stereochemical identities.

As further confirmation of the above argument, X-ray crystallographic data for compound **9g trans** was obtained (crystallized from hexane/DCM). Piperazine **8g cis** did not crystallize. The Boc protecting group of **8g cis** was removed, the free amine recovered and converted to its HCl salt **10** which afforded crystals suitable for X-ray crystallography (Scheme 4). In both cases the X-ray data confirmed assignments made on the basis of the above described NMR studies (Figure 5).



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Scheme 4. Preparation of compound **10** for X-ray crystallography.

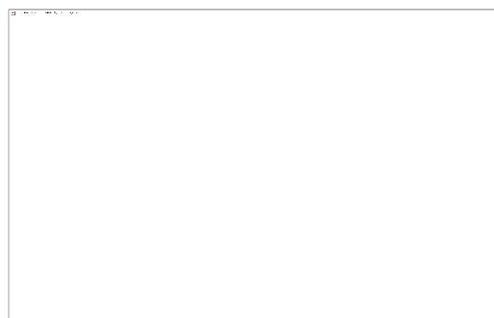
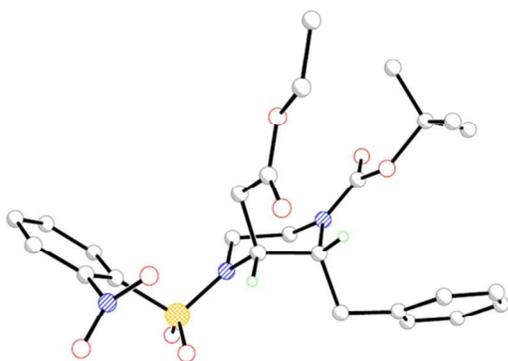


Figure 5. Structures of **9g trans** and **10** determined by X-ray crystallography.

Key to our design of generating both diastereomers, with the exception of the branched Val and Leu cases, virtually no diastereoselectivity of the synthetic sequence shown in Scheme 2 was observed across the range of amino acids used. Both Val and Leu displayed variability in both the dr of the aza-Michael addition of ethanolamine to their acrylates **3c/3h** and **3d/5h** and in the appearance of intermediates **5c/5h** and **5d/5j** in the ¹H NMR spectra. Their derived piperazine scaffolds, however, were identical between enantiomers. Table 1 shows the results obtained.



Entry #	R	PG	dr <i>cis/trans</i>	Amount Produced (g)(<i>cis</i>)	Amount produced (g)(<i>trans</i>)	er (%:%) [*] <i>cis/trans</i>
1	(S)-CH ₃ 8a <i>cis</i>/9a <i>trans</i>	Boc	1.1:1	7.7	7.0	>99.8:0.2/>99.8:0.2
2	(R)-CH ₃ 8f <i>cis</i>/9f <i>trans</i>	Boc	1.1:1	7.0	6.2	>99.8:0.2/>99.8:0.2
3	(S)-CH ₂ Ph 8b <i>cis</i>/9b <i>trans</i>	Boc	1.2:1	7.3	6.1	98.9:1.1/>99.8:0.2
4	(R)-CH ₂ Ph 8g <i>cis</i>/9g <i>trans</i>	Boc	1.3:1	9.5	7.3	>99.8:0.2/98.5:1.5
5	(S)- <i>i</i> Bu 8c <i>cis</i>/9c <i>trans</i>	Boc	1.1:1	6.4	5.6	>99.8:0.2/97.9:2.1
6	(R)- <i>i</i> Bu 8h <i>cis</i>/9h <i>trans</i>	Boc	2.2:1	3.7	1.7	>99.8:0.2/97.4:2.6
7	(S)- <i>i</i> Pr 7d <i>cis</i>/7d <i>trans</i>	H	1:1.1	2.8	3.0	>99.8:0.2/>99.8:0.2
8	(R)- <i>i</i> Pr 7j <i>cis</i>/7j <i>trans</i>	H	1:1.8	0.16	0.30	>99.8:0.2/>99.8:0.2
9	(R)- CH ₂ OCH ₂ Ph [§] 7e <i>cis</i>/7e <i>trans</i>	H	1.5:1	4.5	3.1	95.4:4.6/97.9:2.1 [#]
10	(S)- CH ₂ OCH ₂ Ph [§] 7k <i>cis</i>/7k <i>trans</i>	H	1.3:1	1.4	1.1	97.8:2.2/97.9:2.1 [#]

Table 1. Scaffolds synthesized via Scheme 2 and 3. ^{*}er = enantiomeric ratio %retention:%inversion. [§]the stereochemical designation of the carbon bearing the benzyloxymethyl sidechain changes from (S) to (R) upon cyclization to the piperazine. Therefore, (S) Ser gives (R) **7e *cis/trans*** and (R) Ser gives (S) **7k *cis/trans***. [#]the observed er reflected the er of the starting material serine O-benzyl ether. See SI for chiral analytical data.

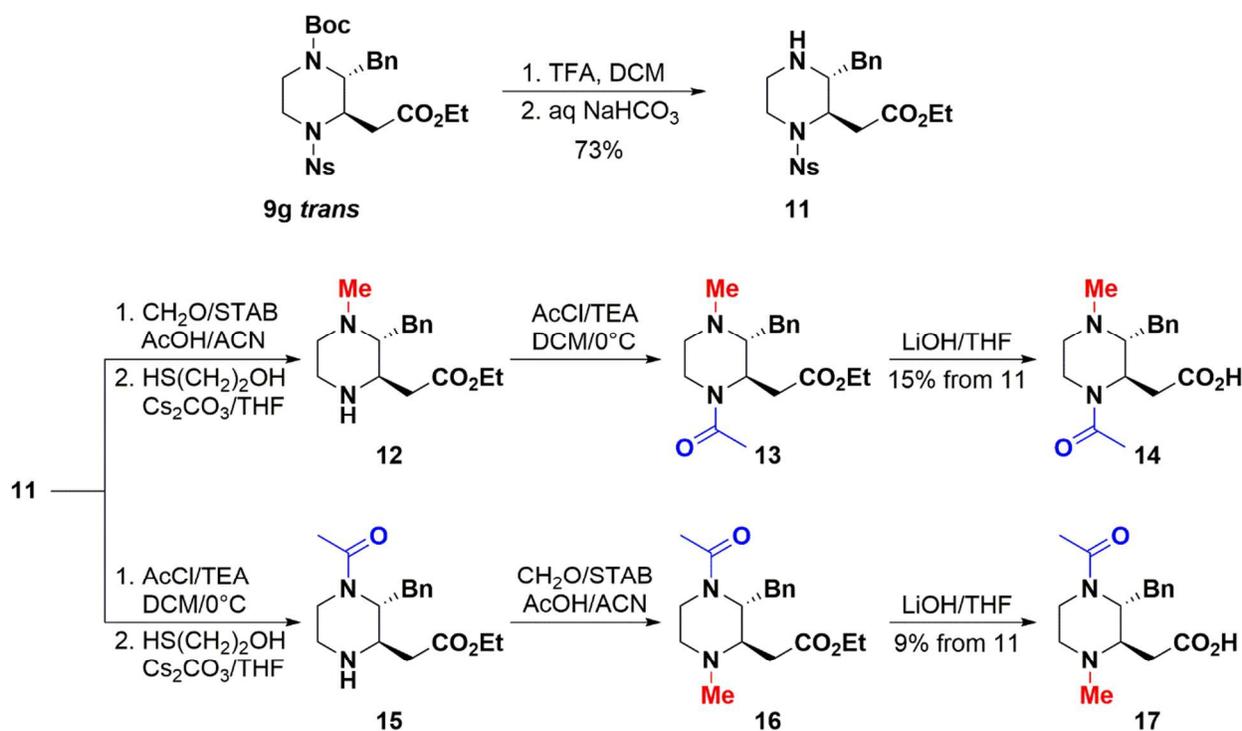
Chiral analysis was carried out by HPLC. The piperazine scaffolds were synthesized in racemic form according to the sequence in Scheme 2. For each racemate, the diastereomers were separated by column chromatography and then subjected to chiral HPLC analysis. After optimal conditions were found to separate the racemate into its component antipodes, the enantiomerically enriched scaffolds were analyzed using these same conditions to determine the enantiomeric ratio (er). From this analysis, it was demonstrated that the stereointegrity was well preserved in all cases.

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3 The ability of the piperazines to access a variety of conformations while presenting the same
4 regiospecific substituents is a key advantage of systematic chemical diversity. Of the four
5 possible conformers shown for the two diastereomers, three are populated in solution at rt
6 (Figure 3). The introduction of either sp^2 or sp^3 character independently to N_1 and N_4 will further
7 perturb the conformations of the scaffolds. This change in hybridization can thus add an
8 additional element of conformational diversity by placing the substituents in unique orientations
9 with respect to the piperazine ring. Generating all possible stereoisomers as shown in Table 1
10 (and the corresponding stereoisomers of the regioisomeric 2,6 and 2,5 family branches) the
11 alternative modes of N diversification will create a set of analogs that will immediately provide
12 deep insight into SAR against target proteins.
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16 Finally, the overarching goal for producing these piperazine scaffolds was for their combinatorial
17 diversification and integration into small-molecule screening libraries. Rather than being limited
18 to the truncated chemical space that is accessible by following the traditional N_1 - N_4
19 diversification paradigm, we have expanded the reach of these piperazines by incorporating, at
20 the outset, the stereochemical diversity offered by one of the four saturated carbon centers. The
21 production of libraries can therefore proceed in a combinatorial fashion by substitution on N_1
22 and N_4 to produce scores of novel drug-like frameworks.
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26 Using scaffold **9g trans** as an exemplar, Scheme 5 depicts the alternative modes of orthogonal
27 diversification that we envision for our scaffolds using the examples of methyl (which imparts sp^3
28 character) and acetyl (which imparts sp^2 character) as the diversity elements. Removal of the
29 Boc protecting group from **9g trans** affords the enantiomerically pure common intermediate **11**.
30 Reductive methylation at N_4 is followed by unmasking of N_1 to give **12**. Treatment of **12** with
31 AcCl affords the bis-diversified ester **13**. Subsequent hydrolysis gives the final piperazine acetic
32 acid **14**. Reversal of the order of diversification ultimately gives the alternative regioisomerically
33 substituted **17**.
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37 The stereochemical diversity represented within these scaffolds in concert with the synthetic
38 capability to derivatize the two nitrogen atoms to introduce sp^2 or sp^3 character generates the
39 additional feature of conformational diversity; this will provide an extended ability to controllably
40 populate conformational micro-environments that are not attainable in sp^2 -enriched compounds.
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Scheme 5. Alternative *N*-diversification sequences for scaffold **9g trans**.

Conclusion

In this report, we disclose the use of systematic chemical diversity to guide the synthesis of a 20-member branch of 3-substituted piperazine-2-acetic acid esters, most of them on multigram scale, as part of a family of piperazine acetic acid esters. The resulting piperazine scaffolds are poised for their use as starting materials for compound library production by combinatorial synthesis. The strategy involved an intermolecular aza-Michael reaction on an acrylate to furnish a chiral 1,2-diamine followed by an S_N2 reaction to form the piperazine ring. It is noteworthy that this pathway is distinct from our previously reported intramolecular aza-Michael reaction to form the 2,6-piperazines which was found to be unproductive in this instance. This illustrates that systematic chemical diversity may require unique synthetic strategies toward the generation of complete scaffold families. The work described here completes a second branch of the piperazine scaffold family. Consequently, the synthesis of the final 2,5-piperazine branch is currently in progress and will be described in a future report.

Experimental

The abbreviation “Ns” refers exclusively to 2-nitrophenylsulfonyl.

General Methods. All starting materials and reagents were purchased from commercial sources and used without further purification. Solvents were purchased as either anhydrous grade products in sealed containers or reagent grade and used as received. All reactions were carried out in dry glassware under a nitrogen atmosphere using standard disposable or gastight

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3 syringes, disposable or stainless steel needles, cannula, and septa. Stirring was achieved with
4 magnetic stir bars or with an overhead mechanical stirrer. Flash column chromatography was
5 performed with SiO₂ (230-400 mesh) or by using an automated chromatography instrument with
6 an appropriately sized column. Thin layer chromatography was performed on silica gel 60F₂₅₄
7 plates (E. Merck). Non-UV active compounds were visualized on TLC using one of the following
8 stains: KMnO₄, ninhydrin, *p*-anisaldehyde, PMA, 2,4-DNP or bromocresol green. ¹H and ¹³C
9 NMR spectra were recorded on an instrument operating at either 600MHz or 800MHz, and
10 150MHz or 200MHz respectively. IR spectra were obtained neat on an FT-IR instrument and
11 are expressed in cm⁻¹. LCMS data were collected using an HPLC instrument coupled to a low
12 resolution mass spectrometer with single quadrupole ionization operating in either positive or
13 negative ion mode. The analytical method utilized a C₁₈ column (2.1×50mm, 1.8μm) eluting with
14 a linear gradient of 95%/5% water/CH₃CN (modified with 0.05% formic acid; T=0min
15 flow=0.35mL/min) to 95%/5% CH₃CN/water (T=3.5min flow= 0.5mL/min) then 95%/5%
16 CH₃CN/water to T=5min (0.5ml/min). Peak detection was done at 254nm and 230nm for UV
17 active compounds. For non-UV active compounds total ion count was used. High-resolution
18 mass spectrometry (HRMS) spectra were obtained on a Thermo Scientific Q Exactive hybrid
19 quadrupole-Orbitrap mass spectrometer equipped with a HESI source and using lock masses
20 for correction. Samples were introduced into the HRMS via reversed phase HPLC on an
21 Accucore Vanquish C18+ column (2.1×100mm, 1.5μm) eluting with a linear gradient of 95%/5%
22 water/acetonitrile (modified with 0.1% formic acid) to 10%/90% water/acetonitrile over 8 min.
23 Chiral HPLC analysis of piperazines was carried out on an instrument with automated 6-column
24 array (Daicel Chiralpak I-series, IA through IF, 4.6×150mm, 5μm). Racemates were screened
25 using a heptane (A)/ethanol (B) gradient (flow=1mL/min) as follows: T=0min (%A/%B) 95/5,
26 T=1min 95/5, T=11min 10/90 (linear gradient), T=13min 10/90, T=13.1min 95/5, T=15min 95/5.
27 Racemates that gave unsatisfactory resolution of enantiomers were rescreened using the above
28 gradient with *i*PrOH instead of EtOH. Additional editing of the gradient to optimize separation
29 was carried out as needed. Chiral materials were analyzed using optimized conditions and their
30 traces were compared to the racemic traces for determination of enantiomeric ratio (er).
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39 To determine our limit of detection (LOD) by chiral HPLC, 5μL aliquots of 2mg/mL stocks (10ng
40 injections) of purified protected scaffolds were subjected to chiral HPLC analysis to resolve and
41 quantify enantiomers and determine enantiomeric ratios. Scouting solvent conditions across six
42 4.6mm x 150mm chiral columns (ChiralPak IA-IE) identified chromatographic conditions for
43 every compound that successfully effected baseline resolution of the enantiomers of the
44 racemic products. Absorbance at 254nm (nosyl or benzyl) was used to detect and quantify the
45 amount of scaffold. Serial dilutions of the 2mg/mL stocks established that our LOD is
46 approximately 0.02ng/injection, or 0.2% of the total material loaded under these conditions.
47 Therefore the limit of our detection of enantiomeric ratio is ≥99.8:0.2. All chiral traces of the
48 scaffolds are included in the Supplementary Information.
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51 Automated preparative reverse-phase HPLC purification was performed using a Mass Directed
52 Fractionation (MDF) system with UV-DAD detection and a single quadrupole mass
53 spectrometer. The instrument used a C₁₈ column (21.2mm i.d. × 150mm, 5 μm, w/19mm ×
54 10mm guard column). The crude product was dissolved in methanol and purified utilizing an
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3 elution of water (modified with 0.05% formic acid) and methanol, with a linear gradient
4 increasing from 5% to 100% methanol over 15 m at a flow rate of 20 mL/min. Purification was
5 carried out by mass trigger only.
6

7 Where indicated ^1H NMR peak assignments were made using COSY, NOESY, HSQC and
8 HMBC protocols. All chemical shifts are quoted on the δ scale and were referenced to residual
9 non-deuterated solvent as an internal standard. Signal multiplicities are described using the
10 following abbreviations: s = singlet, d = doublet, t = triplet, b = broad, quar = quartet, quin =
11 quintet, m = multiplet, v = very; abbreviations are combined, e.g. vbs = very broad singlet. The
12 NMRs of products isolated as unseparated mixtures of diastereomers are included for reference
13 only.
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16 **Scheme 2 (Representative Procedures)**

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20 *Tert*-butyl (*S*)-(1-hydroxy-3-phenylpropan-2-yl)carbamate (2b). A 2000mL rb flask was
21 equipped with a stir bar, rubber septum and nitrogen inlet. The flask was charged with (*N-tert*-
22 butoxycarbonyl)-*S*-phenylalanine (50.0 g, 188.46 mmol) followed by dry THF (100 mL). The
23 mixture was stirred until dissolution was complete. The solution was cooled to 0° C in an
24 ice/water bath. $\text{BH}_3\cdot\text{THF}$ (1M, 380 mL, 380 mmol, 2.0 eq) was added by cannula. The reaction
25 was stirred for 3h at 0° C. The septum and nitrogen inlet were removed and brine (125 mL) was
26 added very slowly until outgassing ceased, then more rapidly afterward. Water (200 mL) was
27 added followed by EtOAc (300 mL). The two-phase mixture was transferred to a separatory
28 funnel and partitioned. The aqueous was extracted with EtOAc (300 mL) and discarded. The
29 combined organic was dried over MgSO_4 , filtered and evaporated *in vacuo* to a colorless oil
30 (44.05 g, 93% crude yield). The crude alcohol was used without further purification (Note: in
31 some cases the crude alcohol was observed to contain a white solid, possibly boron salts.
32 These were removed by an additional water wash.) Crude yields were 60%-98% across the
33 range of substrates. ^1H NMR (600 MHz, CDCl_3) δ 7.32 (m, 2H), 7.25 (m, 3H), 4.81 (vbd, J = 6.0
34 Hz, 1H), 3.89 (vbs, 1H), 3.68 (dd, J = 11.0, 3.8 Hz, 1H), 3.57 (dd, J = 11.1, 5.3 Hz, 1H), 2.86 (d,
35 J = 7.3 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (151 MHz, CDCl_3) δ 156.2, 137.8, 129.3, 128.6, 126.5,
36 79.7, 64.4, 53.7, 37.5, 28.4. IR: 3350, 2984, 1684, 1526, 1314, 1268. HRMS (HESI-TOF) m/z
37 calc for $(\text{M}+\text{H})^+$ ($\text{C}_{14}\text{H}_{22}\text{NO}_3$) $^+$ 252.1600, found 252.1595.
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43 The following additional intermediates were prepared using the method described for 2b.
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46 *Tert*-butyl (*S*)-(1-hydroxypropan-2-yl)carbamate (2a). 32 g, colorless oil, 69% crude yield. ^1H
47 NMR (600 MHz, CDCl_3) δ 4.74 (vbd, J = 7.3 Hz, 1H), 3.87 – 3.71 (m, 1H), 3.64 (dd, J = 11.8, 5.2
48 Hz, 1H), 3.55 – 3.45 (m, 1H), 2.90 (vbs, 1H), 1.45 (s, 9H), 1.15 (d, J = 6.8 Hz, 3H). ^{13}C NMR
49 (151 MHz, CDCl_3) δ 156.4, 79.7, 67.3, 48.6, 28.4, 17.3. IR: 3337, 2975, 1682, 1515, 1365, 1247,
50 1163. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_8\text{H}_{18}\text{NO}_3$) $^+$ 176.1287, found 176.1283.
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53 *Tert*-butyl (*S*)-(1-hydroxy-4-methylpentan-2-yl)carbamate (2c). 32 g, colorless oil, 79% crude
54 yield. ^1H NMR (600 MHz, CDCl_3) δ 4.64 (d, J = 7.9 Hz, 1H), 3.79 – 3.72 (bm, 1H), 3.67 – 3.65
55 (m, 1H), 3.51 (dd, J = 11.0, 6.0 Hz, 1H), 1.67 (dqvar, J = 13.4, 6.7 Hz, 1H), 1.46 (s, 9H), 1.34 –
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3 1.28 (m, 2H), 0.94 (dd, $J = 6.6, 2.7$ Hz, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 156.6, 79.6, 66.5,
4 62.7, 51.0, 40.6, 34.9, 28.4, 24.8, 23.0, 22.2, 18.9, 13.8. IR: 3332, 2955, 2360, 1683, 1508,
5 1365, 1275, 1165. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{11}\text{H}_{24}\text{NO}_3$) $^+$ 218.1756, found
6 218.1751.
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9 *Tert*-butyl (*S*)-(1-hydroxy-3-methylbutan-2-yl)carbamate (2d). 32 g, colorless oil, 98% crude
10 yield. ^1H NMR (600 MHz, CDCl_3) δ 4.76 (vbd, $J = 8.6$ Hz, 1H), 3.81 (vbd, $J = 6.2$ Hz, 1H), 3.71 –
11 3.59 (m, 2H), 3.46 – 3.42 (m, 1H), 1.84 (dt, $J = 13.4, 6.8$ Hz, 1H), 1.45 (s, 9H), 0.96 (d, $J = 6.8$
12 Hz, 3H), 0.94 (d, $J = 6.7$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 156.9, 79.6, 64.2, 58.1, 29.3,
13 28.4, 19.5, 18.5. IR: 3338, 2963, 1684, 1507, 1390, 1365, 1246, 1165. HRMS (HESI-TOF) m/z
14 calc for $(\text{M}+\text{H})^+$ ($\text{C}_{10}\text{H}_{22}\text{NO}_3$) $^+$ 204.1600, found 204.1597.
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18 *Tert*-butyl (*R*)-(1-(benzyloxy)-3-hydroxypropan-2-yl)carbamate (2e). 46.5 g, colorless oil, 97%
19 crude yield. ^1H NMR (600 MHz, CDCl_3) δ 7.39 – 7.31 (m, 5H), 4.55 (AB, $J = 14.6, 2.7$ Hz, 2H),
20 3.83 – 3.76 (m, 2H), 3.72 – 3.64 (m, 3H), 1.46 (s, 9H). ^{13}C NMR (151 MHz, CDCl_3) δ 156.1,
21 137.7, 128.5, 127.9, 127.7, 79.7, 73.5, 70.7, 64.0, 51.6, 28.4. IR: 3332, 2980, 2359, 1685, 1497,
22 1365, 1275, 1260, 1164. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{15}\text{H}_{24}\text{NO}_4$) $^+$ 282.1705, found
23 282.1700.
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27 *Tert*-butyl (*R*)-(1-hydroxypropan-2-yl)carbamate (2f). 43 g, colorless oil, 92% crude yield. ^1H
28 NMR (600 MHz, CDCl_3) δ 4.72 (t, $J = 7.8$ Hz, 1H), 3.78 – 3.74 (m, 1H), 3.63 (dt, $J = 11.4, 3.1$
29 Hz, 1H), 3.50 (dd, $J = 11.0, 6.1$ Hz, 1H), 2.90 (vbs, 1H), 1.45 (s, 9H), 1.15 (d, $J = 6.8$ Hz, 3H).
30 ^{13}C NMR (151 MHz, CDCl_3) δ 156.4, 79.7, 67.3, 48.6, 28.4, 17.3. IR: 3350, 2980, 2934, 1677,
31 1522, 1445, 1249, 1071. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_8\text{H}_{18}\text{NO}_3$) $^+$ 176.1287, found
32 176.1282.
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36 *Tert*-butyl (*R*)-(1-hydroxy-3-phenylpropan-2-yl)carbamate (2g). 36.5 g, colorless oil, 79% crude
37 yield. ^1H NMR identical to 2b. ^{13}C NMR (151 MHz, CDCl_3) δ 156.2, 137.8, 129.3, 128.5, 126.5,
38 79.7, 64.4, 53.7, 37.4, 28.4. IR: 3350, 2984, 2938, 1684, 1526, 1442, 1365, 1314, 1268, 1250,
39 1166. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{14}\text{H}_{22}\text{NO}_3$) $^+$ 252.1600, found 252.1595.
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42 *Tert*-butyl (*R*)-(1-hydroxy-4-methylpentan-2-yl)carbamate (2h). 33 g, colorless oil, 85% crude
43 yield. ^1H NMR identical to 2c. ^{13}C NMR (201 MHz, CDCl_3) δ 156.6, 79.6, 66.8, 51.1, 40.5, 28.4,
44 24.8, 23.0, 22.2. IR: 3325, 2955, 2870, 1685, 1508, 1366, 1275, 1260, 1168. HRMS (HESI-
45 TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{11}\text{H}_{24}\text{NO}_3$) $^+$ 218.1756, found 218.1752.
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48 *Tert*-butyl (*R*)-(1-hydroxy-3-methylbutan-2-yl)carbamate (2j). 31 g, colorless oil, 60% crude
49 yield. ^1H NMR identical to 2d. ^{13}C NMR (151 MHz, CDCl_3) δ 156.9, 79.6, 64.2, 58.1, 29.3, 28.4,
50 19.5, 18.5. IR: 3332, 2963, 1684, 1506, 1390, 1365, 1246, 1165. HRMS (HESI-TOF) m/z calc
51 for $(\text{M}+\text{H})^+$ ($\text{C}_{10}\text{H}_{22}\text{NO}_3$) $^+$ 204.1600, found 204.1597.
52

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54 *Tert*-butyl (*S*)-(1-(benzyloxy)-3-hydroxypropan-2-yl)carbamate (2k). 8.2 g, colorless oil, 86%
55 crude yield. ^1H NMR identical to 2e. ^{13}C NMR (151 MHz, CDCl_3) δ 156.1, 137.6, 128.5, 127.9,
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3 127.7, 79.7, 73.5, 70.7, 64.0, 51.6, 28.4. IR: 3338, 3005, 2979, 2359, 1685, 1497, 1365, 1275,
4 1260, 1164. HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{15}H_{24}NO_4$)⁺ 282.1705, found 282.1699.
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7 **Ethyl (S,E)-4-((tert-butoxycarbonyl)amino)-5-phenylpent-2-enoate (3b).** A 2000mL 3-neck
8 rb flask was equipped with a mechanical stirrer and a nitrogen inlet. The flask was charged with
9 a solution of crude *tert*-butyl (S)-(1-hydroxy-3-phenylpropan-2-yl)carbamate 2b (40.00 g, 159.1
10 mmol) in dry DCM (720 mL). Solid NaHCO₃ (40.10 g, 477.5 mmol, 3 eq) was added, followed by
11 DMP (101.20 g, 238.60 mmol, 1.50 eq). The reaction mixture was stirred at rt for 2h and
12 followed by TLC (30% EtOAc/hex). The reaction mixture was cooled to 0° C and treated with aq
13 Na₂S₂O₃ solution (50% w/v, 200 mL) followed by sat aq NaHCO₃ (100 mL) and water (200 mL).
14 The two phase mixture was vigorously stirred for 15m. Stirring was stopped and the phases
15 were allowed to separate. The lower DCM phase was drawn from the flask by siphon. The flask
16 was charged with DCM (600 mL). Stirring was resumed for 5m, then stopped. The lower organic
17 phase was drawn from the flask by siphon. The aq phase was drawn from the flask by siphon
18 and discarded. The combined organic phase was returned to the flask and washed with brine
19 (300 mL). The organic phase was drawn from the flask by siphon, dried over MgSO₄, filtered
20 and evaporated to afford the crude aldehyde as an oil. The aldehyde was immediately used
21 without further characterization.
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26 A 1000 mL rb flask was equipped with a magnetic stir bar and ground glass jointed nitrogen
27 inlet. The flask was charged with a solution of *tert*-butyl (S)-(1-oxo-3-phenylpropan-2-
28 yl)carbamate derived from the above procedure in dry DCM (480 mL). Solid
29 (ethoxycarbonylmethylene)triphenylphosphorane (60.90 g, 175.01 mmol) was added in portions
30 with stirring at rt. The homogenous solution was stirred at rt for 24h. The solution was partially
31 evaporated *in vacuo* to a moderately viscous oil. Solid silica gel was added to the oil and the
32 mixture swirled to effect complete suspension of the silica gel. All volatiles were then removed *in*
33 *vacuo* and the free flowing dry silica gel placed atop a silica gel column hand packed with
34 hexanes. Elution of the column (5-10% EtOAc/hexanes) afforded the title compound as a
35 colorless oil (23.0 g, 45% yield from crude 2b). ¹H NMR (600 MHz, CDCl₃) δ 7.32 (t, *J* = 7.4 Hz,
36 2H), 7.28 – 7.26 (m, 1H), 7.19 (dd, *J* = 7.0, 1.8 Hz, 2H), 6.93 (dd, *J* = 15.7, 5.0 Hz, 1H), 5.88
37 (dd, *J* = 15.7, 1.8 Hz, 1H), 4.64 – 4.56 (m, 2H), 4.20 (quar, *J* = 7.1 Hz, 2H), 2.94 – 2.90 (m, 2H),
38 1.42 (s, 9H), 1.29 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 166.1, 154.9, 147.6, 136.4,
39 129.4, 128.6, 126.9, 121.1, 60.5, 28.3, 14.2. IR: 3351, 2979, 1716, 1688, 1520, 1161. HRMS
40 (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{18}H_{26}NO_4$)⁺ 320.1862, found 320.1856.
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46 The following additional intermediates were prepared using the method described for **3b**.
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48 **Ethyl (S,E)-4-((tert-butoxycarbonyl)amino)pent-2-enoate (3a).** 24 g, colorless oil, 54% yield
49 from crude 2a. ¹H NMR (600 MHz, CDCl₃) δ 6.87 (dd, *J* = 15.8, 4.7 Hz, 1H), 5.90 (dd, *J* = 15.7,
50 1.7 Hz, 1H), 4.61 (d, *J* = 8.4 Hz, 1H), 4.41 (vbs, 1H), 4.20 (dq, *J*_{quar} = 6.5 Hz, *J*_d = 0.6 Hz,
51 2H), 1.45 (s, 9H), 1.29 (d, *J* = 7.1 Hz, 3H), 1.27 (d, *J* = 6.4 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃)
52 δ 166.4, 154.9, 149.4, 120.1, 79.7, 60.4, 47.0, 28.4, 20.3, 14.2. IR: 3349, 2977, 1689, 1515,
53 1366, 1245, 1161, 1045. HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{12}H_{22}NO_4$)⁺ 244.1549, found
54 244.1544.
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Ethyl (S,E)-4-((tert-butoxycarbonyl)amino)-6-methylhept-2-enoate (3c). 27 g, colorless oil, 65% yield from crude 2c. ^1H NMR (600 MHz, CDCl_3) δ 6.87 (dd, $J = 15.6, 5.2$ Hz, 1H), 5.92 (dd, $J = 15.6, 1.5$ Hz, 1H), 4.51 (d, $J = 8.8$ Hz, 1H), 4.38 – 4.31 (m, 1H), 4.19 (dq, $J_{\text{quar}} = 7.1$ Hz, $J_{\text{d}} = 1.5$ Hz, 2H), 1.69 (septet, $J = 6.8$ Hz, 1H), 1.45 (s, 9H), 1.39 (t, $J = 7.3$ Hz, 2H), 1.29 (t, $J = 7.0$ Hz, 3H), 0.94 (t, $J = 6.7$ Hz, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 166.4, 155.1, 148.9, 120.4, 79.6, 60.4, 49.8, 43.9, 28.4, 24.7, 22.7, 22.2, 14.2. IR: 3349, 2958, 1688, 1517, 1366, 1275, 1261, 1161. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{15}\text{H}_{28}\text{NO}_4$) $^+$ 286.2018, found 286.2013.

Ethyl (S,E)-4-((tert-butoxycarbonyl)amino)-5-methylhex-2-enoate (3d). 36 g, colorless oil, 58% yield from crude 2d. ^1H NMR (600 MHz, CDCl_3) δ 6.87 (dd, $J = 15.7, 5.1$ Hz, 1H), 5.93 (dd, $J = 15.6, 1.6$ Hz, 1H), 4.60 (d, $J = 8.1$ Hz, 1H), 4.20 (dq, $J_{\text{quar}} = 7.3, J_{\text{d}} = 2.1$ Hz, 3H), 1.87 (septet, $J = 13.1, 6.6$ Hz, 1H), 1.46 (s, 9H), 1.30 (t, $J = 7.2$ Hz, 3H), 0.95 (d, $J = 6.9$ Hz, 3H), 0.92 (d, $J = 6.8$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 166.3, 155.4, 147.3, 121.5, 79.7, 60.4, 56.7, 32.3, 28.4, 18.8, 18.0, 14.2. IR: 3341, 2966, 1698, 1514, 1366, 1301, 1158. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{14}\text{H}_{26}\text{NO}_4$) $^+$ 272.1862, found 272.1856.

Ethyl (R,E)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)pent-2-enoate (3e). 35 g, colorless oil, 60% yield from crude 2e. ^1H NMR (600 MHz, CDCl_3) δ 7.39 – 7.35 (m, 2H), 7.32 (ddd, $J = 6.1, 2.1, 1.2$ Hz, 3H), 6.95 (dd, $J = 15.7, 5.0$ Hz, 1H), 6.00 (dd, $J = 15.7, 1.8$ Hz, 1H), 4.60 – 4.49 (m, 3H), 4.21 (dq, $J_{\text{quar}} = 7.1$ Hz, $J_{\text{d}} = 1.4$ Hz, 2H), 3.60 (m, 1H), 1.46 (s, 9H), 1.31 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 166.1, 155.2, 146.1, 137.5, 128.5, 127.9, 127.7, 122.0, 79.8, 73.3, 71.3, 60.4, 51.4, 28.4, 14.2. IR: 3349, 2982, 1706, 1496, 1365, 1275, 1260, 1161. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{19}\text{H}_{28}\text{NO}_5$) $^+$ 350.1967, found 350.1964.

Ethyl (R,E)-4-((tert-butoxycarbonyl)amino)pent-2-enoate (3f). 28 g, colorless oil, 46% yield from crude 2f. ^1H NMR identical to **3a**. ^{13}C NMR (151 MHz, CDCl_3) δ 166.4, 154.9, 149.3, 120.2, 79.7, 60.4, 47.0, 28.4, 20.3, 14.2. IR: 3330, 2982, 1718, 1677, 1523, 1366, 1247, 1161. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{12}\text{H}_{22}\text{NO}_4$) $^+$ 244.1549, found 244.1542.

Ethyl (R,E)-4-((tert-butoxycarbonyl)amino)-5-phenylpent-2-enoate (3g). 35 g, colorless oil, 73% yield from crude 2g. ^1H NMR identical to **3b**. ^{13}C NMR (151 MHz, CDCl_3) δ 166.1, 154.9, 147.6, 136.4, 129.4, 128.6, 126.9, 121.1, 79.9, 60.5, 52.3, 40.9, 28.3, 14.2. IR: 3351, 2979, 1716, 1688, 1520, 1366, 1288, 1161. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{18}\text{H}_{26}\text{NO}_4$) $^+$ 320.1862, found 320.1856.

Ethyl (R,E)-4-((tert-butoxycarbonyl)amino)-6-methylhept-2-enoate (3h). 25.6 g, colorless oil, 59% yield from crude 2h. ^1H NMR identical to **3c**. ^{13}C NMR (201 MHz, CDCl_3) δ 166.4, 155.1, 148.9, 120.4, 79.7, 60.4, 49.8, 43.9, 28.4, 24.7, 22.7, 22.2, 14.2. IR: 3340, 2958, 1689, 1517, 1366, 1275, 1261, 1165. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{15}\text{H}_{28}\text{NO}_4$) $^+$ 286.2018, found 286.2013.

Ethyl (R,E)-4-((tert-butoxycarbonyl)amino)-5-methylhex-2-enoate (3j). 3.8 g, colorless oil, 56% yield from crude 2j. ^1H NMR identical to **3d**. ^{13}C NMR (151 MHz, CDCl_3) δ 166.3, 155.3,

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3 147.3, 121.5, 79.7, 60.4, 56.7, 32.3, 28.4, 18.8, 18.0, 14.2. IR: 3340, 2966, 1698, 1654, 1515,
4 1231, 1277, 1158. HRMS (HESI-TOF) m/z calc for $(M+H)^+$ $(C_{14}H_{26}NO_4)^+$ 272.1862, found
5 272.1855.
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8 **Ethyl (S,E)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)pent-2-enoate (3k).** 7.3 g,
9 colorless oil, 71% yield from crude 2k. 1H NMR identical to **3e**. ^{13}C NMR (151 MHz, $CDCl_3$) δ
10 166.2, 155.2, 146.1, 137.5, 128.5, 127.9, 127.7, 122.0, 79.9, 73.3, 71.3, 60.5, 51.4, 28.4, 14.3.
11 IR: 3346, 2983, 1703, 1496, 1365, 1275, 1260, 1161. HRMS (HESI-TOF) m/z calc for $(M+H)^+$
12 $(C_{19}H_{28}NO_5)^+$ 350.1967, found 350.1961.
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15 Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)-5-phenylpentanoate (4b).
16 A 1000 mL round bottom flask was equipped with a magnetic stir bar, septum and nitrogen inlet.
17 The flask was charged with a solution of ethyl (S,E)-4-((tert-butoxycarbonyl)amino)-5-
18 phenylpent-2-enoate **3b** (23.00 g, 72.00 mmol) in dry DCM (60 mL). Ethanolamine (17.20 mL,
19 288.00 mmol, 4.00 eq) was added by syringe. The homogenous solution was stirred at rt for
20 48h. The progress of the reaction was followed by TLC (80% EtOAc/hex). The solution was
21 evaporated to an oil, redissolved in EtOAc (200 mL) and washed with brine. The aqueous layer
22 was back extracted with EtOAc (200 mL). The combined organic phases were dried over
23 $MgSO_4$, filtered and evaporated to give the title compound as an oil (22 g, 80% crude yield). The
24 title compound was used without further purification.
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28 The following additional intermediates were prepared using the method described for 4b.
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30 Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)pentanoate (4a). 30 g,
31 colorless oil, 86% crude yield from **3a**.
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34 Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)-6-methylheptanoate (4c).
35 32 g, colorless oil, 95% crude yield from **3c**.
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38 Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)-5-methylhexanoate (4d). 37
39 g, colorless oil, 83% crude yield from **3d**.
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42 Ethyl (4R)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)pentanoate
43 (4e). 39 g, colorless oil, 94% crude yield from **3e**.
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45 Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)pentanoate (4f). 27 g,
46 colorless oil, 77% crude yield from **3f**.
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49 Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)-5-phenylpentanoate (4g).
50 38 g, colorless oil, 91% crude yield from **3g**.
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53 Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)-6-methylheptanoate (4h).
54 31.8 g, colorless oil, 102% crude yield from **3h**.
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Ethyl (4*R*)-4-((*tert*-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)-5-methylhexanoate (4j). 4.5 g, colorless oil, 96% crude yield from **3j**.

Ethyl (4*S*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)pentanoate (4k). 8.5 g, colorless oil, 99% crude yield from **3k**.

Ethyl (4*S*)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (5b). A 1000 mL rb flask was equipped with a magnetic stirring bar and nitrogen inlet. The flask was charged with a solution of crude ethyl (4*S*)-4-((*tert*-butoxycarbonyl)amino)-3-((2-hydroxyethyl)amino)-5-phenylpentanoate **4b** (22.00 g, 57.80 mmol) in dry ACN (225 mL). Solid Na₂CO₃ (36.80 g, 347.00 mmol, 6.0 eq) was added, followed by 2-nitrobenzenesulfonyl chloride (NsCl, 25.60 g, 116.00 mmol, 2.0 eq). The reaction was stirred at 45 °C for 24h. The reaction was cooled to rt and filtered. The filtrate was partitioned between brine (200 mL) and EtOAc (100 mL; Note: it is not necessary to remove acetonitrile if brine is used. No emulsification is observed). The aq phase was extracted with EtOAc (100 mL). The combined organic phases were dried over MgSO₄, filtered and evaporated to an oil. The crude product was chromatographed over silica gel with an EtOAc/hexane gradient (20-40% EtOAc/hexanes) to afford the inseparable title compounds as a yellow oil (22.0 g, 38.90 mmol, 67% yield from crude **4b**). ¹H NMR (600 MHz, CDCl₃) δ 8.17 (dt, *J* = 7.2, 3.0 Hz, 1H), 7.74 – 7.57 (m, 3H), 7.27 – 7.13 (m, 6H), 4.40 – 4.35 (m, 2H), 4.15 – 4.07 (m, 4H), 3.92 (bm, 2H), 3.71 – 3.66 (m, 1H), 3.53 (dt, *J* = 15.6, 5.9 Hz, 1H), 3.21 (dd, *J* = 14.4, 3.7 Hz, 0.66H), 2.76 (bm, 1H), 2.74 (dd, *J* = 16.0, 3.8 Hz, 1.5H), 2.48 (dd, *J* = 16.1, 3.6 Hz, 0.69H), 2.32 (dd, *J* = 14.4, 10.8 Hz, 0.72H), 1.28 – 1.22 (m, 13H). ¹³C NMR (151 MHz, CDCl₃) δ 171.9, 171.2, 170.0, 155.8, 148.1, 137.5, 134.0, 132.5, 131.9, 131.8, 131.61, 131.58, 131.4, 129.5, 129.1, 128.55, 128.45, 128.38, 126.54, 126.46, 125.0, 124.1, 123.9, 79.8, 79.4, 62.4, 61.9, 61.3, 61.1, 60.4, 58.8, 58.6, 54.2, 53.4, 47.9, 47.6, 39.3, 39.0, 36.5, 36.3, 28.12, 28.10, 28.03, 21.0, 14.2, 14.1, 14.0. IR: 2978, 1701, 1543, 1367, 1246, 1160. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₆H₃₆N₃O₉S)⁺ 566.2172, found 566.2170.

The following additional intermediates were prepared using the method described for **5b**.

Ethyl (4*S*)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)pentanoate (5a). 30 g, yellow oil 71% yield from crude **4a**. ¹H NMR (600 MHz, CDCl₃) δ 8.14 – 7.98 (m, 1H), 7.83 – 7.60 (m, 3H), 4.51 (vbs, 1H), 4.28 – 4.03 (m, 3H), 3.90 – 3.62 (m, 3H), 3.50 (t, *J* = 6.0 Hz, 1H), 2.76 – 2.72 (m, 0.62H), 2.62 – 2.48 (m, 1.34H), 1.40 (s, 3H), 1.36 (s, 6H), 1.31 (d, *J* = 4.6 Hz, 1H), 1.28 – 1.14 (m, 4H), 1.05 (bd, *J* = 5.6 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 172.1, 170.9, 170.1, 155.5, 148.0, 133.9, 131.8, 131.70, 131.65, 131.4, 125.0, 124.0, 79.9, 69.9, 62.1, 61.8, 61.3, 61.2, 61.1, 60.4, 59.5, 48.8, 47.9, 47.4, 36.9, 36.5, 28.30, 28.28, 28.22, 19.5, 19.2, 14.2, 14.1, 14.0. IR: 2979, 1701, 1543, 1367, 1159. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₀H₃₂N₃O₉S)⁺ 490.1859, found 490.1856.

Ethyl (4*S*)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)-6-methylheptanoate (5c). 29 g, yellow oil, 59% yield from crude **4c**. ¹H NMR (600 MHz, CDCl₃) δ 8.15 – 8.04 (m, 1H), 7.74 – 7.70 (m, 3H), 7.62 – 7.56 (m, 1H),

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3 4.27 (d, $J = 10.1$ Hz, 1H), 4.18 – 4.07 (m, 3H), 3.92 – 3.77 (m, 4H), 3.59 – 3.49 (m, 3H), 2.75
4 (dd, $J = 16.0, 8.5$ Hz, 1H), 2.55 – 2.51 (m, 0.4H), 2.48 – 2.45 (m, 1.3H), 1.45 – 1.26 (m
5 overlapping s, 12H), 1.26 (m overlapping t, $J = 7.1$ Hz, 6H), 0.90 (m, 3H), 0.79 (d, $J = 6.8$ Hz,
6 3H), 0.76 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 172.2, 155.9, 148.0, 133.8, 131.8,
7 131.62, 131.61, 124.02, 123.95, 79.9, 62.3, 61.9, 61.2, 61.1, 59.2, 51.0, 47.5, 41.9, 39.4, 36.8,
8 35.5, 28.3, 28.3, 24.6, 23.6, 21.1, 19.3, 14.12, 14.05. IR: 2985, 1701, 1543, 1367, 1275, 1260,
9 1160. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{23}\text{H}_{38}\text{N}_3\text{O}_9\text{S}$) $^+$ 532.2329, found 532.2327.
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13 **Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-**
14 **nitrophenyl)sulfonamido)-5-methylhexanoate (5d).** 16 g, yellow oil, 27% yield from crude 4d.
15 ^1H NMR (600 MHz, CDCl_3) δ 8.18 (dd, $J = 7.2, 1.5$ Hz, 1H), 8.03 (d, $J = 9.0$ Hz, 0.7H), 7.76 –
16 7.67 (m, 4H), 7.63 (d, $J = 6.8$ Hz, 0.8H), 7.57 (d, $J = 9.1$ Hz, 0.8H), 5.15 (d, $J = 10.9$ Hz, 0.6H),
17 4.41 – 4.29 (m, 2H), 4.14 – 3.89 (m, 6H), 3.86 – 3.71 (m, 4H), 3.60 (dt, $J = 15.7, 5.1$ Hz, 1H),
18 3.52 – 3.39 (m, 2H), 2.70 – 2.58 (m, 2.5H), 2.49 (d, $J = 6.1$ Hz, 0.6H), 2.36 (dd, $J = 16.8, 2.0$ Hz,
19 1H), 1.88 – 1.82 (m, 1H), 1.37 – 1.19 (m, 22H), 0.96 – 0.87 (m, 8H). ^{13}C NMR (151 MHz, CDCl_3)
20 δ 172.1, 171.2, 171.0, 156.8, 156.5, 148.2, 148.1, 134.0, 133.6, 132.8, 132.3, 132.2, 131.7,
21 131.5, 131.2, 124.1, 123.7, 79.9, 79.7, 62.1, 62.0, 61.3, 60.9, 60.4, 56.9, 56.8, 56.5, 47.8, 36.5,
22 36.4, 29.2, 28.27, 28.26, 26.9, 20.7, 20.2, 15.0, 14.4, 14.2, 14.0. IR: 3427, 2969, 1707, 1544,
23 1367, 1244, 1159. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{22}\text{H}_{36}\text{N}_3\text{O}_9\text{S}$) $^+$ 518.2172, found
24 518.2170.
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29 **Ethyl (4R)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-**
30 **nitrophenyl)sulfonamido)pentanoate (5e).** 15g, yellow oil, 41% yield from crude 4e. See
31 Scheme 3 for separation of **5e syn/5e anti** diastereomers.
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34 **Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-**
35 **nitrophenyl)sulfonamido)pentanoate (5f).** 29 g, yellow oil, 66% yield from crude 4f. ^1H NMR
36 (600 MHz, CDCl_3) ^1H NMR identical to **5a**. ^{13}C NMR (151 MHz, CDCl_3) δ 172.1, 171.2, 170.9,
37 170.1, 155.5, 148.2, 148.0, 135.0, 133.9, 132.7, 132.4, 132.0, 131.9, 131.7, 131.64, 131.60,
38 131.5, 125.0, 124.1, 79.9, 61.8, 61.3, 61.2, 61.1, 60.4, 59.6, 48.8, 47.9, 36.9, 28.3, 28.2, 21.0,
39 19.6, 19.2, 14.2, 14.1, 14.0. IR: 2979, 1701, 1543, 1367, 1159. HRMS (HESI-TOF) m/z calc for
40 $(\text{M}+\text{H})^+$ ($\text{C}_{20}\text{H}_{32}\text{N}_3\text{O}_9\text{S}$) $^+$ 490.1859, found 490.1858.
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43 **Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-**
44 **nitrophenyl)sulfonamido)-5-phenylpentanoate (5g).** 32 g, yellow oil, 56% yield from crude
45 4g. ^1H NMR (600 MHz, CDCl_3) ^1H NMR identical to **5b**. ^{13}C NMR (151 MHz, CDCl_3) δ 171.9,
46 170.9, 155.8, 148.1, 137.5, 137.0, 135.2, 134.0, 133.7, 132.54, 132.47, 132.38, 132.32, 131.9,
47 131.8, 131.62, 131.56, 131.45, 129.5, 129.09, 129.05, 128.62, 128.56, 128.45, 128.39, 126.6,
48 126.5, 125.0, 124.4, 124.1, 123.9, 79.8, 79.5, 62.4, 61.9, 61.3, 61.1, 60.4, 58.8, 58.6, 54.2, 53.4,
49 47.9, 47.6, 39.4, 39.0, 36.5, 36.4, 31.6, 28.12, 28.10, 25.3, 22.7, 21.0, 20.7, 14.2, 14.1, 14.0. IR:
50 3376, 2978, 1701, 1543, 1367, 1246, 1160. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$
51 $(\text{C}_{26}\text{H}_{36}\text{N}_3\text{O}_9\text{S})^+$ 566.2172, found 566.2171.
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Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)-6-methylheptanoate (5h). 22 g, yellow oil, 45% yield from crude 4h. ¹H NMR (600 MHz, CDCl₃) δ 8.08 – 7.97 (m, 1H), 7.77 – 7.66 (m, 2H), 7.62 – 7.52 (m, 1H), 5.06 (d, *J* = 10.7 Hz, 1H), 4.31 – 4.19 (m, 1H), 4.06 (dq_{uar}, *J*_{quar} = 7.2 Hz, *J*_d = 2.5 Hz, 2H), 3.98 – 3.83 (m, 1H), 3.80 (ddd, *J* = 10.9, 8.7, 2.9 Hz, 1H), 3.50 (ddd, *J* = 16.0, 5.7, 2.8 Hz, 1H), 3.34 (ddd, *J* = 16.0, 7.1, 2.8 Hz, 1H), 2.72 – 2.52 (m, 2H), 1.50 – 1.43 (m, 1H), 1.42 – 1.38 (m, 2H), 1.30 – 1.24 (m, 1H), 1.23 (t, *J* = 7.2 Hz, 3H), 0.92 (dd, *J* = 12.7, 6.6 Hz, 6H). ¹³C NMR (201 MHz, CDCl₃) δ 171.1, 156.3, 148.3, 133.7, 132.5, 131.6, 131.4, 123.7, 79.6, 62.3, 61.3, 59.7, 51.1, 48.1, 42.9, 36.3, 28.3, 28.2, 28.2, 25.0, 23.8, 21.5, 14.0. IR: 2985, 1701, 1544, 1367, 1275, 1260, 1161, 1024. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₃H₃₈N₃O₉S)⁺ 532.2329, found 532.2322.

Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)-5-methylhexanoate (5j). 1.32 g, yellow oil, 18% yield from crude 4j. ¹H NMR (600 MHz, CDCl₃) δ 8.18 (dd, *J* = 7.2, 1.5 Hz, 1H), 8.03 (d, *J* = 9.0 Hz, 0.7H), 7.76 – 7.67 (m, 4H), 7.63 (d, *J* = 6.8 Hz, 0.8H), 7.57 (d, *J* = 9.1 Hz, 0.8H), 5.15 (d, *J* = 10.9 Hz, 0.6H), 4.41 – 4.29 (m, 2H), 4.14 – 3.89 (m, 6H), 3.86 – 3.71 (m, 4H), 3.60 (dt, *J* = 15.7, 5.1 Hz, 1H), 3.52 – 3.39 (m, 2H), 2.70 – 2.58 (m, 2.5H), 2.49 (d, *J* = 6.1 Hz, 0.6H), 2.36 (dd, *J* = 16.8, 2.0 Hz, 1H), 1.88 – 1.82 (m, 1H), 1.41 (s, 5H), 1.37 (s, 4H), 1.28 – 1.19 (m, 5H), 0.96 – 0.87 (m, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 172.1, 171.2, 171.0, 156.8, 156.5, 148.2, 148.1, 134.0, 133.6, 132.8, 132.3, 132.2, 131.7, 131.5, 131.2, 124.1, 123.7, 79.8, 79.7, 62.1, 62.0, 61.3, 60.9, 60.4, 56.9, 56.8, 56.5, 47.8, 36.5, 36.4, 29.2, 28.27, 28.26, 26.9, 20.7, 20.2, 15.0, 14.4, 14.2, 14.0. IR: 3427, 2969, 1708, 1544, 1367, 1244, 1159, 1024. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₂H₃₆N₃O₉S)⁺ 518.2172, found 518.2170.

Ethyl (4S)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)pentanoate (5k). 4.7 g, yellow oil, 38% yield from crude 4k. See Scheme 3 for separation of **5k syn/5k anti** diastereomers.

Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)-5-phenylpentanoate (6b). A 500 mL rb flask was equipped with a magnetic stir bar, rubber septum and nitrogen inlet. The flask was charged with a solution of ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)-5-phenylpentanoate **5b** (22.0 g, 38.89 mmol) in dry DCM (200 mL). The flask was cooled to –78° C. TEA (6.51 mL, 46.7 mmol, 1.20 eq) was added by syringe, followed by MsCl (3.61 mL, 46.7 mmol, 1.20 eq). The reaction was stirred at –78° C for 1h. Complete conversion to the mesylate was confirmed by TLC (20% EtOAc/hex). The cold reaction was poured into a separatory funnel followed by saturated aq NaHCO₃ (200 mL). The layers were separated and the organic washed once with brine (200 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give the title compound as a yellow oil. The compounds were immediately used without further purification or characterization.

The following additional intermediates were prepared using the method described for 6b. In all cases TLC data (20% EtOAc/hex, double elution) indicated 100% conversion of alcohols **5a-d**,

f-j to their corresponding mesylates. The crude products, all yellow oils, were used without further characterization and under the assumption of quantitative yield.

Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)pentanoate (6a).

Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-6-methyl-3-((N-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)heptanoate (6c).

Ethyl (4S)-4-((tert-butoxycarbonyl)amino)-5-methyl-3-((N-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)hexanoate (6d).

Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-((methylsulfonyl)oxy)ethyl)-4-nitrophenyl)sulfonamido)pentanoate (6f).

Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-((methylsulfonyl)oxy)ethyl)-4-nitrophenyl)sulfonamido)-5-phenylpentanoate (6g).

Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-6-methyl-3-((N-(2-((methylsulfonyl)oxy)ethyl)-4-nitrophenyl)sulfonamido)heptanoate (6h).

Ethyl (4R)-4-((tert-butoxycarbonyl)amino)-5-methyl-3-((N-(2-((methylsulfonyl)oxy)ethyl)-4-nitrophenyl)sulfonamido)hexanoate (6j).

Ethyl 2-((3S)-3-benzyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7b). A 500 mL rb flask was equipped with a magnetic stir bar and nitrogen inlet. The flask was charged with a solution of ethyl (4S)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)-5-phenylpentanoate 6b (assuming 100% conversion of **5b** to 6b) in dry DCM (100 mL). The solution was cooled to 0°C and was treated with TFA (17.85 mL, 233.34 mmol, 6 eq). The solution was stirred for 16h as it slowly warmed in the cold bath. The reaction progress was followed by TLC (30% EtOAc/hexanes) and LCMS analysis of samples withdrawn from the reaction and partitioned between aq NaHCO₃ and EtOAc. Toluene (20 mL) was added and the solution evaporated *in vacuo* to an oil. Toluene (20 mL) was again added and swirled to effect dissolution, then evaporated to an oil. The oil was dissolved in EtOAc (150 mL) and, with stirring, treated dropwise with sat aq NaHCO₃ (200 mL) until basic. The two-phase mixture was vigorously stirred at rt for 1h. The reaction was partitioned and the aq was extracted with EtOAc (2 x 100 mL). The combined EtOAc layers were washed with brine, dried over MgSO₄, filtered and evaporated to an oil to afford an inseparable mixture of diastereomers **7b** as a yellow oil (16g, 75% crude yield). The crude product was taken forward into the next step without further purification (Note: diastereomers **7d** and **7j** could be separated by chromatography over silica gel (40% EtOAc/hexanes). LCMS *m/z* calc for (M+H)⁺ (C₂₁H₂₆N₃O₆S)⁺ 448.2, found 448.1.

The following additional intermediates were prepared using the method described for 7b.

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3 Ethyl 2-((3S)-3-methyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7a). 22.0 g, 96% crude
4 yield from **5a**. LCMS m/z calc for $(M+H)^+$ ($C_{15}H_{22}N_3O_6S$)⁺ 372.1, found 372.1.
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7 Ethyl 2-((3S)-3-isobutyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7c). 20.0 g, 88% crude
8 yield from **5c**. LCMS m/z calc for $(M+H)^+$ ($C_{18}H_{28}N_3O_6S$)⁺ 414.2, found 414.1.
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10 **Ethyl 2-((2R,3S)-3-isopropyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7d cis**, the
11 more retained isomer). Isolated by chromatography (40% EtOAc/hexanes). 2.8 g, 22% yield
12 from **5d**. ¹H NMR (600 MHz, CDCl₃) δ 8.12 (dd, $J = 5.2, 1.4$ Hz, 1H), 7.77 – 7.59 (m, 3H), 4.49
13 (dt, $J = 8.3, 3.8$ Hz, 1H), 3.90 (dq_{quar}, $J_{quar} = 7.2$ Hz, $J_d = 3.5$ Hz, 2H), 3.81 (dt, $J_d = 13.8$ Hz, $J_t =$
14 1.4 Hz, 1H), 3.30 (dt, $J_t = 13.2$ Hz, $J_d = 2.9$ Hz, 1H), 3.02 (bd, $J = 11.8$ Hz, 1H), 2.80
15 (overlapping dd, $J = 15.3, 8.9$ Hz, 2H), 2.51 (dd, $J = 15.2, 4.5$ Hz, 1H), 2.44 (d, $J = 9.8$ Hz, 1H),
16 1.36 (dpent, $J = 9.7, 6.6$ Hz, 1H), 1.18 (dt, $J_t = 7.2$ Hz, $J_d = 0.9$ Hz, 3H), 1.00 (d, $J = 6.7$ Hz, 3H),
17 0.94 (d, $J = 6.5$ Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 171.4, 147.6, 134.3, 133.2, 131.7, 131.4,
18 124.0, 65.7, 60.7, 53.5, 47.0, 41.5, 31.2, 29.3, 19.7, 18.7, 14.0. IR: 3004, 2988, 1728, 1541,
19 1275, 1260, 1159. Chiral analysis on ChiralPak IE (heptane/*i*PrOH). $[\alpha]_D^{25} -4.96$ (c 5.0, EtOH).
20 HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{17}H_{26}N_3O_6S$)⁺ 400.1542, found 400.1533.
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24 **Ethyl 2-((2S,3S)-3-isopropyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7d trans**, the
25 less retained isomer). 3.0 g, 24% yield from **5d**. ¹H NMR (600 MHz, CDCl₃) δ 8.11 (dt, $J_t = 6.0$
26 Hz, $J_d = 1.5$ Hz, 1H), 7.79 – 7.61 (m, 3H), 4.35 (dt, $J_d = 10.3$ Hz, $J_t = 1.5$ Hz, 1H), 4.10 (dq_{quar},
27 $J_{quar} = 7.1, J_d = 2.6$ Hz, 2H), 3.78 (dt, $J_d = 10.9$ Hz, $J_t = 2.0$ Hz, 1H), 3.31 (dd, $J = 16.2, 10.2$ Hz,
28 1H), 3.24 – 3.13 (m, 1H), 3.04 (dt, $J_t = 12.3$ Hz, $J_d = 3.6$ Hz, 1H), 2.69 (ddd, $J = 11.9, 3.5, 1.6$
29 Hz, 1H), 2.42 (dd, $J = 16.2, 2.9$ Hz, 1H), 2.16 (dd, $J = 10.5, 1.4$ Hz, 1H), 2.01 (dpent, $J_{pent} = 10.3$
30 Hz, $J_d = 6.6$ Hz, 1H), 1.24 (t, $J = 7.2$ Hz, 3H), 0.87 (d, $J = 6.7$ Hz, 3H), 0.82 (d, $J = 6.5$ Hz, 3H).
31 ¹³C NMR (151 MHz, CDCl₃) δ 171.3, 147.6, 133.6, 131.8, 131.3, 124.3, 61.0, 60.6, 50.6, 41.8,
32 39.3, 35.4, 24.6, 19.9, 18.9, 14.2. IR: 3004, 2988, 1728, 1541, 1275, 1260, 1158. Chiral analysis
33 on ChiralPak IA (heptane/EtOH). $[\alpha]_D^{25} -4.62$ (c 5.0, EtOH) HRMS (HESI-TOF) m/z calc for
34 $(M+H)^+$ ($C_{17}H_{26}N_3O_6S$)⁺ 400.1542, found 400.1532.
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40 Ethyl 2-((3R)-3-methyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7f). 20 g, 90% crude
41 yield from **5f**. LCMS m/z calc for $(M+H)^+$ ($C_{15}H_{22}N_3O_6S$)⁺ 372.1, found 372.1.
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44 Ethyl 2-((3R)-3-benzyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7g). 24 g, 77% crude
45 yield from **5g**. LCMS m/z calc for $(M+H)^+$ ($C_{21}H_{26}N_3O_6S$)⁺ 448.2, found 448.2.
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48 Ethyl 2-((3R)-3-isobutyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7h). 20 g, 83% crude
49 yield from **5h**. LCMS m/z calc for $(M+H)^+$ ($C_{18}H_{28}N_3O_6S$)⁺ 414.2, found 414.1.
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51 **Ethyl 2-((2S,3R)-3-isopropyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7j cis**, the
52 more retained isomer). Isolated by chromatography (40% EtOAc/hexanes). 160 mg, 32% yield
53 from **5j**. ¹H NMR identical to **7d cis**. ¹³C NMR (151 MHz, CDCl₃) δ 171.4, 147.6, 134.3, 133.2,
54 131.7, 131.5, 124.0, 65.7, 60.7, 53.5, 47.0, 41.5, 31.2, 29.3, 19.7, 18.7, 14.0. IR: 3004, 2988,
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1728, 1541, 1275, 1260, 1159. Chiral analysis on ChiralPak IE (heptane/*i*PrOH). $[\alpha]_D^{25} +4.66$ (c 5.0, EtOH) HRMS (HESI-TOF) m/z calc for (M+H)⁺ (C₁₇H₂₆N₃O₆S)⁺ 400.1542, found 400.1539.

Ethyl 2-((2*R*,3*R*)-3-isopropyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7j trans, the less retained isomer). 302 mg, 60% yield from **5j**. ¹H NMR identical to **7d trans**. ¹³C NMR (151 MHz, CDCl₃) δ 171.3, 147.7, 133.5, 131.8, 131.4, 124.3, 61.1, 60.6, 50.6, 41.8, 39.3, 35.4, 24.6, 19.9, 18.9, 14.2. IR: 3004, 2988, 1728, 1541, 1275, 1260, 1159. Chiral analysis on ChiralPak IA (heptane/EtOH). $[\alpha]_D^{25} +4.43$ (c 5.0, EtOH) HRMS (HESI-TOF) m/z calc for (M+H)⁺ (C₁₇H₂₆N₃O₆S)⁺ 400.1542, found 400.1533.

Tert-butyl (2*S*,3*R*)-2-benzyl-3-(2-ethoxy-2-oxoethyl)-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (8b cis) and **Tert-butyl (2*S*,3*S*)-2-benzyl-3-(2-ethoxy-2-oxoethyl)-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (9b trans)**. A 500mL rb flask was equipped with a magnetic stir bar. The flask was charged with a solution of the mixture of diastereomers ethyl 2-((3*S*)-3-benzyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate **7b** (16.00g, 35.75mmol) in THF (180 mL). Water (90 mL) was added followed by Na₂CO₃ (11.35g, 107.25mmol) and Boc₂O (27.30g, 125.13mmol). The reaction was followed by TLC (30% EtOAc/hexane). Stirring at rt for 24h produced a clear solution with a small amount of separated water at the bottom of the flask. The mixture was partitioned between EtOAc (200 mL) and more water (200 mL). The layers were separated and the aq extracted again with EtOAc (200 mL). The aq was discarded and the organic was dried over MgSO₄, filtered and evaporated to an oil. The crude products were chromatographed over silica gel with an EtOAc/hexane gradient (10%-30% EtOAc/hexane) to afford the two title compounds as yellow oils.

8b cis (the more retained isomer). 5.7 g, 29% yield from crude **7b**. ¹H NMR (600 MHz, CDCl₃) δ 7.94 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.77 – 7.59 (m, 3H), 7.33 – 7.25 (m, 3H), 7.25 – 7.20 (m, 1H), 7.20 – 7.10 (m, 2H), 4.25 (dt, *J*_t = 7.2, *J*_d = 3.4 Hz, 1H), 4.19 (dt, *J*_t = 9.2 Hz, *J*_d = 3.4 Hz, 1H), 4.05 (two overlapping dquart, *J*_{quar} = 7.1 Hz, *J*_d = 3.5 Hz, 2H), 3.79 – 3.68 (m, 2H), 3.62 – 3.54 (m, 1H), 3.52 – 3.45 (m, 1H), 3.14 (dd, *J* = 14.2, 9.4 Hz, 1H), 2.97 – 2.90 (m, 2H), 2.82 (dd, *J* = 16.0, 6.8 Hz, 1H), 1.26 – 1.24 (overlapping t and s, 12H). ¹³C NMR (151 MHz, CDCl₃) δ 170.3, 154.1, 147.8, 137.9, 134.2, 133.6, 132.0, 130.5, 129.1, 128.5, 126.5, 124.4, 80.3, 61.1, 58.3, 58.0, 45.8, 41.6, 34.5, 33.8, 28.0, 14.1. IR: 3009, 2986, 1735, 1681, 1543, 1360, 1277, 1260, 1160. Chiral analysis on ChiralPak ID (heptane/*i*PrOH). $[\alpha]_D^{25} -1.30$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for (M+H)⁺ (C₂₆H₃₄N₃O₈S)⁺ 548.2067, found 548.2061.

9b trans (the less retained isomer). 6.4 g, 35% yield from crude **7b**. ¹H NMR (600 MHz, CDCl₃) δ 8.13 – 8.08 (m, 1H), 7.76 – 7.68 (m, 3H), 7.28 – 7.15 (m, 3H), 7.11 – 7.06 (m, 2H), 4.49 (t, *J* = 7.6 Hz, 1H), 4.38 (dd, *J* = 9.3, 5.5 Hz, 1H), 4.24 – 4.22 (m, 1H), 4.18 – 4.17 (m, 1H), 4.10 (quar, *J* = 7.1 Hz, 2H), 3.94 – 3.90 (m, 2H), 3.37 – 3.30 (m, 1H), 3.18 (dt, *J*_t = 7.1 Hz, *J*_d = 3.5 Hz, 1H), 2.90 – 2.82 (m, 2H), 2.65 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.44 (dd, *J* = 16.4, 3.0 Hz, 1H), 1.25 – 1.15 (two singlets overlapping m, 12H). ¹³C NMR (151 MHz, CDCl₃) δ 170.4, 170.2, 154.7, 154.6, 147.7, 137.6, 137.1, 133.93, 133.88, 133.20, 133.19, 132.0, 131.7, 131.5, 129.21, 129.16, 129.09, 128.5, 128.4, 128.2, 126.5, 126.4, 124.5, 124.4, 80.4, 80.2, 60.89, 60.87, 55.5, 54.1, 52.8, 52.2, 40.9, 39.1, 37.1, 35.6, 35.5, 35.1, 34.7, 28.2, 27.9, 14.1, 14.0. IR: 3004, 2987, 1731, 1688, 1542, 1364, 1275, 1260, 1160. Chiral analysis on ChiralPak ID (heptane/EtOH). $[\alpha]_D^{25}$

–1.26 (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{26}H_{34}N_3O_8S$) $^+$ 548.2067, found 548.2060.

The following additional piperazines were prepared using the above described procedure. In all cases the *cis* isomers were the more retained and the *trans* isomers were the less retained by silica gel chromatography (10%-30% EtOAc/hexanes).

Cis (8a, 8c, 8f-8h) the more retained isomers

***Tert*-butyl (2*S*,3*R*)-3-(2-ethoxy-2-oxoethyl)-2-methyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (8a *cis*)**. 8.0 g, yellow oil, 28% yield from crude 7a. 1H NMR (600 MHz, $CDCl_3$) δ 8.07 (ddd, $J = 5.3, 2.9, 1.4$ Hz, 1H), 7.73 – 7.68 (m, 3H), 4.23 (dt, $J_t = 7.0$ Hz, $J_d = 3.6$ Hz, 1H), 4.05 – 4.01 (m, 3H), 3.69 – 3.42 (m, 4H), 2.82 (dd, $J = 16.2, 7.2$ Hz, 1H), 2.66 (ddd, $J = 16.3, 6.8, 0.8$ Hz, 1H), 1.46 (d, $J = 0.9$ Hz, 9H), 1.30 (dd, $J = 7.0, 1.0$ Hz, 3H), 1.23 (dt, $J_t = 6.7$ Hz, $J_d = 1.0$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 154.4, 147.9, 134.2, 133.6, 131.9, 130.6, 124.4, 80.5, 61.0, 57.9, 52.9, 45.3, 41.7, 34.4, 28.4, 14.2, 14.0. IR: 3004, 2987, 1732, 1687, 1543, 1365, 1275, 1260, 1161. Chiral analysis on ChiralPak IF (heptane/EtOH). $[\alpha]_D^{25} -0.83$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{20}H_{30}N_3O_8S$) $^+$ 472.1754, found 472.1751.

***Tert*-butyl (2*S*,3*R*)-3-(2-ethoxy-2-oxoethyl)-2-isobutyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (8c *cis*)**. 6.4 g, yellow oil, 25% yield from crude 7c. 1H NMR (600 MHz, $CDCl_3$) δ 8.06 – 8.04 (m, 1H), 7.72 – 7.66 (m, 3H), 4.25 (dt, $J_t = 7.0$ Hz, $J_d = 3.5$ Hz, 1H), 4.06 – 3.99 (two overlapping dquar, $J_{quar} = 7.2$ Hz, $J_d = 3.6$ Hz, 2H), 3.95 (ddd, $J = 10.6, 4.5, 3.4$ Hz, 1H), 3.62 – 3.46 (m, 4H), 2.79 (dd, $J = 16.2, 7.3$ Hz, 1H), 2.68 (dd, $J = 16.2, 6.8$ Hz, 1H), 1.90 (ddd, $J = 14.1, 10.7, 4.4$ Hz, 1H), 1.61 – 1.52 (m, 1H), 1.45 (s, 9H), 1.22 (two overlapping t, $J = 7.1$ Hz, 4H), 0.92 (d, $J = 6.6$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 154.6, 147.9, 134.2, 133.6, 131.9, 130.6, 124.4, 80.5, 61.0, 58.0, 55.8, 45.0, 36.7, 34.4, 28.3, 24.6, 23.4, 21.8, 14.1. IR: 3004, 2986, 1732, 1686, 1543, 1365, 1275, 1260, 1161. Chiral analysis on ChiralPak IE (heptane/EtOH). $[\alpha]_D^{25} -1.67$ (c 5.0, EtOH) HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{23}H_{36}N_3O_8S$) $^+$ 514.2223, found 514.2217.

Tert*-butyl (2*R*,3*S*)-3-(2-ethoxy-2-oxoethyl)-2-methyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (8f *cis*)**. 7.0 g, yellow oil, 27% yield from crude 7f. 1H NMR (600 MHz, $CDCl_3$). 1H NMR identical to **8a *cis. ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 154.4, 147.9, 134.2, 133.6, 131.9, 130.6, 124.4, 80.5, 61.0, 57.9, 52.9, 45.3, 41.7, 34.4, 28.4, 14.2, 14.0. IR: 3004, 2987, 1732, 1687, 1543, 1365, 1275, 1260, 1161. Chiral analysis on ChiralPak IF (heptane/EtOH). $[\alpha]_D^{25} +0.79$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{20}H_{30}N_3O_8S$) $^+$ 472.1754, found 472.1750.

Tert*-butyl (2*R*,3*S*)-2-benzyl-3-(2-ethoxy-2-oxoethyl)-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (8g *cis*)**. 9.5 g, yellow oil, 28% yield from crude 7g. 1H NMR (600 MHz, $CDCl_3$). 1H NMR identical to **8b *cis. ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.3, 154.1, 147.8, 137.9, 134.2, 133.6, 132.0, 130.5, 129.17, 128.5, 126.5, 124.4, 80.3, 61.1, 58.3, 58.0, 45.8, 41.6, 34.5, 33.8, 28.0, 14.1. IR: 3004, 2987, 1731, 1688, 1542, 1364, 1275, 1260, 1160. Chiral analysis on

ChiralPak ID (heptane/*i*PrOH). $[\alpha]_D^{25} +1.22$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{26}H_{34}N_3O_8S$) $^+$ 548.2067, found 548.2064.

Tert-butyl (2*R*,3*S*)-3-(2-ethoxy-2-oxoethyl)-2-isobutyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (8*h cis*). 3.7 g, yellow oil, 33% yield from crude 7*h*. 1H NMR (600 MHz, $CDCl_3$). 1H NMR identical to **8*c cis***. ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.4, 154.6, 147.8, 134.1, 133.6, 131.9, 130.6, 124.4, 80.5, 61.0, 58.0, 55.8, 45.0, 36.7, 34.4, 28.3, 24.6, 23.4, 21.8, 14.1. IR: 3005, 2988, 2360, 2341, 1731, 1686, 1544, 1365, 1275, 1260, 1163. Chiral analysis on ChiralPak IE (heptane/EtOH). $[\alpha]_D^{25} +1.70$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{23}H_{36}N_3O_8S$) $^+$ 514.2223, found 514.2222.

Trans (9*a*, 9*c*, 9*f*-9*h*) the less retained isomers

Tert-butyl (2*S*,3*S*)-3-(2-ethoxy-2-oxoethyl)-2-methyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (9*a trans*). 7.0 g, yellow oil, 25% yield from crude 7*a*. 1H NMR (600 MHz, $CDCl_3$) δ 8.10 (t, $J = 7.0$ Hz, 1H), 7.75 – 7.69 (m, 3H), 4.24 – 4.23 (m, 1H), 4.15 – 4.04 (m, 4H), 3.86 – 3.84 (m, 1H), 3.30 – 3.12 (overlapping dpent, $J_{pent} = 12.7$ Hz, $J_d = 2.8$ Hz, 2H), 2.86 – 2.76 (overlapping dd, $J = 10.6, 7.0$ Hz, 2H total), 2.45 (dt, $J_t = 11.0$ Hz, $J_d = 3.3$ Hz, 1H), 1.47 (s, 3H), 1.44 (s, 6H) 1.27 (two overlapping t, $J = 7.1$ Hz, 3H total), 1.07 (d, $J = 6.8$ Hz, 2H), 1.02 (d, $J = 6.8$ Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 154.8, 147.6, 133.8, 133.3, 131.9, 131.2, 124.4, 124.3, 80.4, 60.9, 60.8, 54.4, 54.0, 49.6, 48.7, 40.9, 40.8, 38.4, 36.8, 35.1, 34.7, 28.3, 28.2, 15.6, 15.1, 14.1, 14.0. IR: 3004, 2987, 1731, 1688, 1542, 1364, 1275, 1260, 1161, 1125. Chiral analysis on ChiralPak IF (heptane/EtOH). $[\alpha]_D^{25} -0.86$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{20}H_{30}N_3O_8S$) $^+$ 472.1754, found 472.1751.

Tert-butyl (2*S*,3*S*)-3-(2-ethoxy-2-oxoethyl)-2-isobutyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (9*c trans*). 5.6 g, yellow oil, 22% yield from crude 7*c*. 1H NMR (600 MHz, $CDCl_3$) δ 8.11 (dd, $J = 7.3, 1.8$ Hz, 1H), 7.76 – 7.69 (m, 3H), 4.21 – 4.07 (m, 5H), 3.91 – 3.80 (m, 1H), 3.23 – 3.02 (m, 2H), 2.85 (dd, $J = 16.5, 10.8$ Hz, 0.75H), 2.78 (dd, $J = 16.5, 9.7$ Hz, 0.25H), 2.46 (dd, $J = 16.6, 3.1$ Hz, 1H), 1.46 (s, 7H), 1.43 (s, 2H), 1.37 – 1.12 (m, 7H), 0.81 (d, $J = 6.4$ Hz, 3H), 0.76 (d, $J = 6.4$ Hz, 2H), 0.72 (d, $J = 6.5$ Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 154.8, 147.6, 133.8, 133.3, 132.0, 131.5, 131.3, 124.4, 80.4, 60.9, 52.7, 51.9, 51.1, 40.78, 40.75, 38.7, 38.5, 38.3, 37.0, 28.3, 28.2, 24.6, 24.4, 22.6, 22.4, 22.3, 14.2, 14.1. IR: 3004, 2986, 1732, 1686, 1543, 1365, 1275, 1260, 1161. Chiral analysis on ChiralPak IE (heptane/EtOH). $[\alpha]_D^{25} -1.69$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{23}H_{36}N_3O_8S$) $^+$ 514.2223, found 514.2222.

Tert-butyl (2*R*,3*R*)-3-(2-ethoxy-2-oxoethyl)-2-methyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (9*f trans*). 6.2 g, yellow oil, 24% from crude 7*f*. 1H NMR (600 MHz, $CDCl_3$). 1H NMR identical to **9*a trans***. ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 154.8, 147.6, 133.8, 133.3, 131.9, 131.2, 124.4, 124.3, 80.4, 60.9, 60.8, 54.4, 54.0, 49.6, 48.7, 40.9, 40.8, 38.4, 36.8, 35.1, 34.7, 28.3, 28.2, 15.6, 15.1, 14.1, 14.0. IR: 3004, 2987, 1732, 1688, 1543, 1365, 1275, 1260, 1161, 1122. Chiral analysis on ChiralPak IF (heptane/EtOH). $[\alpha]_D^{25} +0.87$ (c 5.0, EtOH). HRMS (HESI-TOF) m/z calc for $(M+H)^+$ ($C_{20}H_{30}N_3O_8S$) $^+$ 472.1754, found 472.1753.

Tert-butyl (2R,3R)-2-benzyl-3-(2-ethoxy-2-oxoethyl)-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (9g trans). 7.3 g, yellow oil, 24% yield from crude 7g. ¹H NMR (600 MHz, CDCl₃). ¹H NMR identical to **9b trans**. ¹³C NMR (151 MHz, CDCl₃) δ 170.4, 170.2, 154.65, 154.59, 147.7, 137.6, 137.1, 133.93, 133.88, 133.21, 133.19, 132.0, 131.7, 131.5, 129.2, 129.1, 128.4, 128.3, 126.5, 126.4, 124.5, 124.4, 80.4, 80.2, 60.89, 60.87, 55.5, 54.1, 52.8, 52.2, 40.9, 39.1, 37.1, 35.6, 35.5, 35.1, 34.7, 28.2, 27.9, 14.1, 14.0. IR: 3004, 2987, 1731, 1688, 1542, 1364, 1275, 1260, 1161, 1125. Chiral analysis on ChiralPak ID (heptane/EtOH). [α]_D²⁵ +1.23 (c 5.0, EtOH). HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₆H₃₄N₃O₈S)⁺ 548.2067, found 548.2061.

Tert-butyl (2R,3R)-3-(2-ethoxy-2-oxoethyl)-2-isobutyl-4-((2-nitrophenyl)sulfonyl)piperazine-1-carboxylate (9h trans). 1.4 g, yellow oil, 12% from crude 7h. ¹H NMR (600 MHz, CDCl₃). ¹H NMR identical to **9c trans**. ¹³C NMR (151 MHz, CDCl₃) δ 170.6, 154.8, 147.6, 133.8, 133.3, 131.9, 131.3, 124.4, 80.4, 60.9, 52.7, 51.9, 51.1, 40.8, 40.8, 38.7, 38.5, 38.3, 37.0, 28.3, 28.2, 24.6, 24.4, 22.6, 22.4, 22.3, 14.2, 14.1. IR: 3005, 2988, 2360, 1730, 1687, 1366, 1275, 1260, 1164. [α]_D²⁵ +1.72 (c 5.0, EtOH). HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₃H₃₆N₃O₈S)⁺ 514.2223, found 514.2222.

Scheme 3

Ethyl (3R,4R)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)pentanoate (5e syn, the less retained isomer). The title compound was obtained from compounds 5e (Scheme 2) by column chromatography (10-40% EtOAc/hexanes). 8.5g, yellow oil, 26% yield from crude 4e. ¹H NMR (600 MHz, CDCl₃) δ 8.15 (d, *J* = 8.1 Hz, 1H), 7.62 – 7.56 (m, 3H), 7.37 (dd, *J* = 8.5, 0.9 Hz, 2H), 7.31 (dd, *J* = 6.3, 1.0 Hz, 3H), 4.95 (d, *J* = 9.8 Hz, 1H), 4.64 – 4.55 (m, 1H), 4.40 – 4.33 (m, 2H), 4.13 – 4.02 (m, 2H), 3.85 – 3.81 (m, 2H), 3.72 (dd, *J* = 9.5, 2.7 Hz, 1H), 3.65 (dt, *J* = 15.6, 4.7 Hz, 1H), 3.49 – 3.42 (m, 2H), 2.78 (dd, *J* = 16.3, 8.9 Hz, 1H), 2.50 (bd, *J* = 16.7 Hz, 1H), 1.42 (s, 9H), 1.23 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 171.6, 155.9, 148.0, 138.0, 133.8, 132.5, 131.7, 128.4, 127.6, 127.5, 123.9, 80.0, 72.9, 68.7, 61.9, 60.9, 52.3, 36.2, 28.3, 14.1. IR: 3004, 2987, 1707, 1543, 1367, 1275, 1260, 1160. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₇H₃₈N₃O₁₀S)⁺ 596.2278, found 596.2269.

Ethyl (3S,4R)-5-(benzyloxy)-4-((tert-butoxycarbonyl)amino)-3-((N-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)pentanoate (5e anti, the more retained isomer). The title compound was obtained from compounds 5e (Scheme 2) by column chromatography (10-40% EtOAc/hexanes). 6.5g, yellow oil, 20% yield from crude 4e. ¹H NMR (600 MHz, CDCl₃) δ 8.02 (d, *J* = 8.1 Hz, 1H), 7.68 (dt, *J*_t = 7.7 Hz, *J*_d = 1.3 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 5.0 Hz, 4H), 7.35 – 7.30 (m, 1H), 5.39 (bd, *J* = 10.3 Hz, 1H), 4.62 (td, *J* = 8.3, 5.6 Hz, 1H), 4.55 (½ AB, *J* = 11.7 Hz, 1H), 4.50 (½ AB, *J* = 11.7 Hz, 1H), 4.01 (dt, *J*_t = 7.1 Hz, *J*_d = 3.5 Hz, 3H), 3.89 – 3.79 (m, 2H), 3.58 (t, *J* = 3.4 Hz, 2H), 3.46 – 3.42 (m, 2H), 2.62 (dq_{uar}, *J*_{quar} = 15.0 Hz, *J*_d = 6.7 Hz, 2H), 1.39 (s, 9H), 1.21 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 170.8, 155.9, 148.2, 137.6, 133.6, 132.6, 131.6, 131.5, 128.4, 127.9, 127.8, 123.8, 80.0, 73.3, 70.4, 62.3, 61.2, 56.3, 52.3, 48.2, 36.1, 28.3, 14.0. IR: 3004, 2987, 1707, 1543, 1367, 1275, 1260, 1160. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₇H₃₈N₃O₁₀S)⁺ 596.2278, found 596.2275.

Ethyl (3*R*,4*R*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)pentanoate (**6e syn**). The title compound was obtained using the method described for compound 6b (Scheme 2) with compound **5e syn** as the starting material.

Ethyl (3*S*,4*R*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)pentanoate (**6e anti**). The title compound was obtained using the method described for compound 6b (Scheme 2) with compound **5e anti** as the starting material.

Ethyl 2-((2*R*,3*R*)-3-((benzyloxy)methyl)-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7e cis**)**. 4.5 g, yellow oil, 66% yield from **5e syn**. The title compound was obtained using the method described for compound 7b (Scheme 2) with compound 6e *syn* as the starting material. ¹H NMR (600 MHz, CDCl₃) δ 8.11 (dt, *J*_t = 3.3 Hz, *J*_d = 1.6 Hz, 1H), 7.71 – 7.69 (m, 1H), 7.68 – 7.65 (m, 1H), 7.39 – 7.34 (m, 2H), 7.33 – 7.29 (m, 3H), 4.47 (s, 2H), 4.41 (dt, *J*_t = 6.9, *J*_d = 1.7 Hz, 1H), 3.96 (quar, *J* = 7.1 Hz, 2H), 3.80 (dd, *J* = 13.6, 1.6 Hz, 1H), 3.50 – 3.47 (m, 1H), 3.25 (dt, *J*_t = 12.8 Hz, *J*_d = 3.1 Hz, 1H), 3.19 – 3.16 (m, 2H), 3.00 (dd, *J* = 11.9, 1.2 Hz, 1H), 2.84 (ddd, *J* = 12.1, 3.5, 1.4 Hz, 1H), 2.79 (dd, *J* = 16.0, 7.0 Hz, 1H), 2.58 (dd, *J* = 16.0, 5.9 Hz, 1H), 1.17 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 171.0, 147.6, 137.6, 133.9, 133.5, 131.8, 131.4, 128.5, 127.9, 127.8, 124.2, 73.5, 70.7, 60.8, 58.1, 52.4, 46.0, 42.3, 32.5, 14.0. IR: 3004, 2988, 1726, 1541, 1368, 1275, 1260, 1164, 1124. Chiral analysis on ChiralPak IC (heptane/*i*PrOH). [α]_D²⁵ –3.39 (c 5.0, EtOH) HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₂H₂₈N₃O₇S)⁺ 478.1648, found 478.1638.

Ethyl 2-((2*S*,3*R*)-3-((benzyloxy)methyl)-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7e trans**)**. 3.1 g, yellow oil, 59% yield from **5e anti**. The title compound was obtained using the method described for compound 7b (Scheme 2) with compound 6e *anti* as the starting material. ¹H NMR identical to **7k trans**. ¹³C NMR (151 MHz, CDCl₃) δ 171.1, 147.4, 138.0, 133.5, 131.9, 131.3, 128.4, 127.7, 127.6, 124.3, 73.1, 67.1, 60.8, 53.6, 50.5, 41.8, 39.0, 35.1, 14.1. IR: 3004, 2988, 1727, 1541, 1368, 1275, 1260, 1164, 1124. Chiral analysis on ChiralPak IF (heptane/*i*PrOH). [α]_D²⁵ –3.38 (c 5.0, EtOH) HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₂H₂₈N₃O₇S)⁺ 478.1648, found 478.1639.

Ethyl (3*S*,4*S*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)pentanoate (5k syn**, the less retained isomer)**. The title compound was obtained from compounds 5k (Scheme 2) by column chromatography (10-40% EtOAc/hexanes). 2.3g, 19% yield from crude 4k. ¹H NMR identical to **5e syn**. ¹³C NMR (151 MHz, CDCl₃) δ 171.6, 155.9, 148.0, 137.9, 133.8, 132.5, 131.7, 128.4, 127.6, 127.5, 123.9, 80.0, 72.9, 68.7, 62.0, 60.9, 52.3, 36.2, 28.3, 14.1. IR: 3004, 2987, 1707, 1543, 1367, 1275, 1260, 1160. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₂₇H₃₈N₃O₁₀S)⁺ 596.2278, found 596.2276.

Ethyl (3*R*,4*S*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-hydroxyethyl)-2-nitrophenyl)sulfonamido)pentanoate (5k anti**, the more retained isomer)**. The title compound was obtained from compounds 5k (Scheme 2) by column chromatography (10-40%

EtOAc/hexanes). 2.4g, 20% yield from crude 4k. ^1H NMR identical to **5e anti**. ^{13}C NMR (151 MHz, CDCl_3) δ 170.8, 155.9, 148.2, 137.6, 133.6, 132.6, 131.6, 131.5, 128.4, 127.9, 127.8, 123.8, 80.0, 73.3, 70.4, 62.3, 61.2, 56.3, 52.3, 36.1, 28.3, 14.0. IR: 3004, 2987, 1707, 1543, 1367, 1275, 1260, 1160, 1018. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{27}\text{H}_{38}\text{N}_3\text{O}_{10}\text{S}$) $^+$ 596.2278, found 596.2273.

Ethyl (3*S*,4*S*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)pentanoate (**6k syn**). The title compound was obtained using the method described for compound 6b (Scheme 2) with compound **5k syn** as the starting material.

Ethyl (3*R*,4*S*)-5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-3-((*N*-(2-((methylsulfonyl)oxy)ethyl)-2-nitrophenyl)sulfonamido)pentanoate (**6k anti**). The title compound was obtained using the method described for compound 6b (Scheme 2) with compound **5k anti** as the starting material.

Ethyl 2-((2*S*,3*S*)-3-((benzyloxy)methyl)-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7k cis**)**. 1.4 g, yellow oil, 75% yield from **5k syn**. The title compound was obtained using the method described for compound 7b (Scheme 2) with compound 6e *syn* as the starting material. ^1H NMR identical to **7e cis**. ^{13}C NMR (151 MHz, CDCl_3) δ 171.0, 147.6, 137.6, 133.9, 133.5, 131.8, 131.4, 128.5, 127.9, 127.8, 124.2, 73.5, 70.7, 60.8, 58.1, 52.4, 46.0, 42.3, 32.5, 14.0. IR: 3004, 2988, 1727, 1541, 1368, 1275, 1260, 1164, 1124. Chiral analysis on ChiralPak IC (heptane/*i*PrOH). $[\alpha]_{\text{D}}^{25}$ +3.65 (c 5.0, EtOH) HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{22}\text{H}_{28}\text{N}_3\text{O}_7\text{S}$) $^+$ 478.1648, found 478.1643.

Ethyl 2-((2*R*,3*S*)-3-((benzyloxy)methyl)-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (7k trans**)**. 1.1g, yellow oil, 57% yield from **5k anti**. The title compound was obtained using the method described for compound 7b (Scheme 2) with compound 6e *anti* as the starting material. ^1H NMR (600 MHz, CDCl_3) δ 8.05 (dd, $J = 7.9, 1.3$ Hz, 1H), 7.67 (ddd, $J = 7.8, 7.2, 1.3$ Hz, 1H), 7.64 – 7.57 (m, 2H), 7.37 – 7.33 (m, 2H), 7.32 – 7.29 (m, 1H), 7.27 – 7.23 (m, 2H), 4.41 ($\frac{1}{2}$ AB, $J = 11.9$ Hz, 1H), 4.36 ($\frac{1}{2}$ AB, $J = 12.0$ Hz, 1H), 4.16 (dd, $J = 9.9, 3.2$ Hz, 1H), 4.10 (quar, $J = 7.2$ Hz, 2H), 3.80 (dt, $J_{\text{d}} = 12.9$ Hz, $J_{\text{t}} = 1.7$ Hz, 1H), 3.61 (t, $J = 9.4$ Hz, 1H), 3.33 – 3.22 (m, 2H), 3.12 – 3.04 (m, 2H), 2.71 (dt, $J_{\text{d}} = 10.6$ Hz, $J_{\text{t}} = 1.5$ Hz, 1H), 2.53 (dd, $J = 16.1, 3.4$ Hz, 1H), 1.24 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (151 MHz, CDCl_3) δ 171.1, 147.4, 138.0, 133.5, 131.9, 131.4, 128.4, 127.7, 127.6, 124.3, 73.1, 67.1, 60.8, 53.6, 50.5, 41.8, 39.0, 35.1, 14.1. IR: 3004, 2988, 1726, 1541, 1368, 1275, 1260, 1164, 1124. Chiral analysis on ChiralPak IF (heptane/*i*PrOH). $[\alpha]_{\text{D}}^{25}$ +3.70 (c 5.0, EtOH) HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{22}\text{H}_{28}\text{N}_3\text{O}_7\text{S}$) $^+$ 478.1648, found 478.1645.

Scheme 4

ethyl 2-((2*S*,3*R*)-3-benzyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate hydrochloride (10**)**.

A solution of **8g cis** (30 mg, 0.054 mmol) in DCM (1 mL) was treated with TFA (0.25 mL) at rt. The solution was stirred for 3h at rt. LCMS confirmed complete deprotection. The reaction was

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3 diluted with toluene (2mL) and evaporated *in vacuo* to a residue. The residue was partitioned
4 between EtOAc and sat aq NaHCO₃. The organic was recovered, dried over MgSO₄, filtered
5 and evaporated to a residue. The residue was dissolved in abs EtOH (2mL) and treated with aq
6 2N HCl (0.6 mL). The homogenous solution was evaporated to a residue, then allowed to stand
7 under high vacuum to constant weight. The residue was layered with iPrOAc (3 mL) and placed
8 in a 75° C bath. Anhy EtOH was added dropwise by syringe with swirling until the residue
9 dissolved. The flask was removed from the bath, capped with an inlet adapter and allowed to
10 stand at the back of a fume hood for 6d, affording crystals of the title compound. The
11 supernatant was removed by pipet and the crystals rinsed with hexane (HPLC grade) which was
12 removed by pipet. Residual hexane was allowed to evaporate affording **10**.
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16 **Scheme 5**

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19 The process described below demonstrates the orthogonal diversification of the piperazine
20 scaffolds using techniques suitable for parallel synthesis. No reaction optimization was
21 performed and some intermediates were characterized by LCMS only. MDF purified final
22 products were characterized by HRMS, ¹H and ¹³C NMR.
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25 Ethyl 2-((2*R*,3*R*)-3-benzyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (11). A solution of
26 *tert*-butyl (2*R*,3*R*)-2-benzyl-3-(2-ethoxy-2-oxoethyl)-4-((2-nitrophenyl)sulfonyl)piperazine-1-
27 carboxylate **9g trans** (200mg, 0.365mmol, 1.0eq) in dry DCM (2mL) was treated with TFA (168
28 μL, 2.19 mmol, 6 eq) at rt. The reaction was allowed to stir for 16h. LCMS confirmed complete
29 deprotection. The reaction was diluted with toluene (0.5 mL) and evaporated to a residue. The
30 residue was partitioned between EtOAc and sat aq NaHCO₃. The aqueous was extracted twice
31 with EtOAc. The combined organic layers were washed with brine, dried over anhy Na₂SO₄,
32 filtered and evaporated to afford the title compound. The crude product was used without further
33 purification. LCMS *m/z* calc for (M+H)⁺ (C₂₁H₂₆N₃O₆S)⁺ 448.2, found 448.1.
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37 Ethyl 2-((2*R*,3*R*)-3-benzyl-4-methyl-1-((2-nitrophenyl)sulfonyl)piperazin-2-yl)acetate (12).
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40 A solution of compound 11 (120 mg, 0.268 mmol, 1 eq) in dry ACN (3 mL) was treated with
41 AcOH (38 μL, 0.67 mmol, 2.5 eq) followed by 37% aq HCHO (1.54 mL, 1.60 mmol, 6 eq) at rt.
42 The solution was stirred at rt for 10m, then treated with STAB (170 mg, 0.804 mmol, 3 eq). The
43 reaction was stirred at rt for 16h. LCMS confirmed complete methylation. At the end of this time
44 sat aq NaHCO₃ was slowly added. The mixture was partitioned between EtOAc and sat aq
45 NaHCO₃. The aq was extracted once with EtOAc. The combined organic layers were washed
46 with brine and dried over anhy Na₂SO₄, filtered and evaporated. The crude product was used
47 without further purification. LCMS *m/z* calc for (M+H)⁺ (C₂₂H₂₈N₃O₆S)⁺ 462.2, found 462.2.
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51 A solution of the compound from the above reaction (150 mg crude, 0.325 mmol, 1 eq) in dry
52 THF (2.5 mL) was treated with 2-mercaptoethanol (115 μL, 0.97 mmol, 3 eq based on crude
53 weight) at rt. Cs₂CO₃ (159 mg, 0.48 mmol, 1.5 eq based on crude weight) was added. The
54 reaction was stirred at rt for 16h. LCMS confirmed complete deprotection. The reaction was
55 partitioned between water and EtOAc. The organic layer was dried over anhy MgSO₄, filtered
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and evaporated to a residue. The crude product was purified by normal phase chromatography (5% CH₃OH/DCM) to afford **12** (64 mg, 71% yield). The compound was taken forward to the next step without further purification or characterization. LCMS *m/z* calc for (M+H)⁺ (C₁₆H₂₅N₂O₂)⁺ 277.2, found 277.2.

Ethyl 2-((2*R*,3*R*)-1-acetyl-3-benzyl-4-methylpiperazin-2-yl)acetate (13**).** A 0° C solution of compound **16** (64 mg, 0.231 mmol) in dry DCM (2mL) was treated with Et₃N (48 μL, 0.346 mmol, 1.5eq) followed by AcCl (20 μL, 0.277 mmol, 1.2 eq). The solution was stirred at rt for 2h. LCMS confirmed complete acylation. The reaction mixture was partitioned between DCM and sat aq NaHCO₃. The organic layer was washed with brine, dried over anhy Na₂SO₄, filtered and evaporated to a residue. The crude product was purified by chromatography (5% CH₃OH/DCM) to afford **13** as a pale yellow oil (30mg, 41% yield). ¹H NMR (600 MHz, CDCl₃) δ 7.36 (m, 3H), 7.29 (d, *J* = 6.5 Hz, 1H), 7.27 – 7.19 (m, 4H), 4.35 (t, *J* = 6.7 Hz, 3H), 4.27 (t, *J* = 6.6 Hz, 1H), 4.05 (quar, *J* = 7.1 Hz, 2H), 3.96 (tt, *J* = 7.1, 3.5 Hz, 1H), 3.90 (t, *J* = 5.8 Hz, 2H), 3.80 (t, *J* = 5.9 Hz, 1H), 2.94 (t, *J* = 6.6 Hz, 3H), 2.92 – 2.88 (m, 3H), 2.87 (t, *J* = 5.9 Hz, 1H), 2.19 (s, 2H), 2.09 (s, 4H), 1.99 (s, 3H), 1.16 (t, *J* = 7.1 Hz, 3H), 1.09 (t, *J* = 7.1 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 170.9, 170.8, 170.4, 169.7, 129.5, 129.1, 128.90, 128.85, 63.0, 62.4, 60.8, 60.3, 42.5, 41.7, 37.0, 35.5, 34.1, 29.3, 21.7, 20.87, 20.85, 14.02, 14.01, 13.93. IR: 1730, 1637, 1420, 1378, 1275, 1260. Chiral analysis on ChiralPak IC (heptane/*i*-PrOH). [α]_D²⁵ –0.46 (*c* 5.0, EtOH). HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₁₈H₂₇N₂O₃)⁺ 319.2022, found 319.2019.

2-((2*R*,3*R*)-1-acetyl-3-benzyl-4-methylpiperazin-2-yl)acetic acid (14**).** A solution of compound **13** (30 mg, 0.094 mmol) in dry THF (1.8 mL) was treated with aq LiOH (1M, 230 μL, 0.23 mmol, 2.5 eq). The solution was stirred at rt for 16h. LCMS confirmed complete ester hydrolysis. The reaction mixture was treated with aq HCl (1M, 230 μL). All volatiles were removed. The crude product was purified by automated preparative reverse-phase MDF HPLC as described in the General Methods. The appropriate fractions were evaporated in a parallel evaporator to afford the acid **14** as a clear, colorless oil (12 mg, 44% yield). ¹H NMR (600 MHz, CD₃OD, mixture of conformers and rotamers) δ 7.41 – 7.28 (m, 7H), 5.12 (t, *J* = 7.5 Hz, 1H), 4.65 (dt, *J*_d = 10.9 Hz, *J*_t = 3.5 Hz, 0.5H), 4.28 (t, *J* = 7.3 Hz, 1H), 4.05 (dt, *J*_d = 11.2 Hz, *J*_t = 3.3 Hz, 1H), 3.87 – 3.84 (m, 1H), 3.81 (t, *J* = 6.5 Hz, 2H), 3.75 – 3.70 (m, 2H), 3.65 (dt, *J*_t = 12.3 Hz, *J*_d = 3.6 Hz, 1H), 3.47 (t, *J* = 7.2 Hz, 0.67H), 3.24 – 3.16 (m, 3H), 3.00 (s, 3H), 2.91 (d, *J* = 6.4 Hz, 3H), 2.89 – 2.83 (m, 3H), 2.82 – 2.74 (m, 3H), 2.68 (s, 2H), 2.63 (dd, *J* = 15.7, 6.8 Hz, 1H), 2.38 (t, *J* = 8.1 Hz, 1H), 2.24 (s, 3H), 2.05 (pent, *J* = 7.6 Hz, 0.65H), 1.98 (s, 1.3H). ¹³C NMR (151 MHz, CD₃OD) δ 172.5, 172.1, 171.40, 171.36, 136.0, 129.5, 128.9, 128.8, 128.4, 127.1, 126.8, 64.1, 63.0, 60.8, 59.9, 51.6, 49.4, 45.8, 41.1, 40.9, 40.8, 39.5, 39.1, 35.1, 34.7, 34.6, 33.6, 32.6, 30.3, 28.4, 20.0, 19.5, 17.2. HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ (C₁₆H₂₃N₂O₃)⁺ 291.1709, found 291.1701.

Ethyl 2-((2*R*,3*R*)-4-acetyl-3-benzylpiperazin-2-yl)acetate (**15**).

A 0° C solution of compound **11** (195 mg, 0.435 mmol) in dry DCM (2 mL) was treated with Et₃N (90 μL, 0.652 mmol, 1.5 eq) followed by AcCl (37 μL, 0.522 mmol, 1.2 eq). The solution was stirred at rt for 2h. LCMS confirmed complete acylation. The reaction mixture was partitioned

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3 between DCM and sat aq NaHCO₃. The organic layer was washed with brine, dried over anhy
4 Na₂SO₄, filtered and evaporated to a residue. The crude product was used without further
5 purification. LCMS *m/z* calc for (M+H)⁺ (C₂₃H₂₈N₃O₇S)⁺ 490.2, found 490.1.
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8 A solution of the product from the above reaction (223 mg, 0.435 mmol, 1 eq) in dry THF (3 mL)
9 was treated with 2-mercaptoethanol (160 μL, 1.36 mmol, 3 eq) at rt. Cs₂CO₃ (222 mg, 0.68
10 mmol, 1.5 eq) was added. The reaction was stirred at rt for 16h. LCMS confirmed complete
11 deprotection. The reaction was partitioned between water and EtOAc. The organic layer was
12 dried over anhy MgSO₄, filtered and evaporated to a residue. The crude product was purified by
13 normal phase chromatography (5% CH₃OH/DCM) to afford 15 as a pale yellow oil (90 mg, 65%
14 yield). LCMS *m/z* calc for (M+H)⁺ (C₁₇H₂₅N₂O₃)⁺ 305.2, found 305.2.
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18 **Ethyl 2-((2R,3R)-4-acetyl-3-benzyl-1-methylpiperazin-2-yl)acetate (16).** A solution of
19 compound 15 (90 mg, 0.295 mmol, 1eq) in ACN (2 mL) was treated with AcOH (45 μL, 0.735
20 mmol, 2.5 eq) followed by 37% aq HCHO (1.8 mL, 1.77 mmol, 6 eq) at rt. The solution was
21 stirred at rt for 10m, then treated with STAB (188 mg, 0.885 mmol, 3 eq). The reaction was
22 stirred at rt for 16h. LCMS confirmed complete methylation. At the end of this time sat aq
23 NaHCO₃ was slowly added. The mixture was partitioned between EtOAc and sat aq NaHCO₃.
24 The aq was extracted once with EtOAc. The combined organic layers were washed with brine
25 and dried over anhy Na₂SO₄, filtered and evaporated. The crude product was purified by
26 chromatography (5% CH₃OH/DCM) to afford **16** as a pale yellow oil (29 mg, 28% yield). ¹H
27 NMR (600 MHz, CDCl₃) δ 7.33 – 7.27 (m, 3H), 7.26 – 7.21 (m, 1H), 7.17 (d, *J* = 7.1 Hz, 2H),
28 4.56 – 4.50 (m, 1H), 4.13 – 4.05 (m, 1H), 3.97 (t, *J* = 7.5 Hz, 1H), 3.28 – 3.25 (m, *J* = 1H), 3.17
29 (vbs, 2H), 3.11 – 3.06 (m, 1H), 2.54 – 2.51 (m, 1H), 2.38 (d, *J* = 15.6 Hz, 2H), 1.69 (s, 1H), 1.19
30 (t, *J* = 7.1 Hz, 1H), 1.16 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 170.7, 169.8, 138.4,
31 129.4, 129.2, 128.7, 128.4, 126.8, 60.8, 59.2, 58.7, 47.0, 42.5, 36.7, 21.7, 20.7, 14.1, 14.0. IR:
32 1730, 1637, 1420, 1275, 1260, 1030. Chiral analysis on ChiralPak IC (heptane/*i*-PrOH). [α]_D²⁵
33 +0.44 (*c* 5.0, EtOH) HRMS (HESI-TOF) *m/z* calc for (M+H)⁺ HRMS (HESI-TOF) *m/z* calc for
34 (M+H)⁺ (C₁₈H₂₇N₂O₃)⁺ 319.2022, found 319.2012.
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40 **2-((2R,3R)-4-acetyl-3-benzyl-1-methylpiperazin-2-yl)acetic acid (17).** A solution of **16** (29mg,
41 0.091mmol) in dry THF (1.8 mL) was treated with aq LiOH (1M, 220 μL, 0.220 mmol, 2.5 eq).
42 The solution was stirred at rt for 16h. LCMS confirmed complete ester hydrolysis. The reaction
43 mixture was treated with aq HCl (1M, 220 μL). All volatiles were removed. The crude product
44 was purified by automated preparative reverse-phase MDF HPLC as described in the General
45 Methods. The appropriate fractions were evaporated in a parallel evaporator to afford the acid
46 **17** as a clear, colorless oil (11 mg, 42% yield). ¹H NMR (600 MHz, CD₃OD, mixture of
47 conformers and rotamers). δ 7.31 (t, *J* = 7.4 Hz, 2H), 7.27 – 7.25 (m, 1H), 7.22 – 7.20 (m, 2H),
48 5.00 (t, *J* = 8.0 Hz, 0.35H), 4.50 (dt, *J*_d = 13.9 Hz, *J*_t = 1.4 Hz, 0.62H), 4.17 (dd, *J* = 10.2, 4.7 Hz,
49 0.63H), 3.82 (dt, *J* = 13.6, 2.8 Hz, 0.35H), 3.67 – 3.62 (m, 0.35H), 3.51 – 3.44 (m, 1.29H), 3.41
50 (dd, *J* = 13.7, 10.1 Hz, 0.65H), 3.26 (dt, *J* = 13.0, 4.1 Hz, 0.69H), 3.14 (½ABX, *J* = 13.7, 7.7 Hz,
51 0.36H), 3.08 (½ABX, *J* = 13.6, 8.0 Hz, 0.36H), 3.01 (dd, *J* = 8.7, 4.8 Hz, 0.75H), 2.88 – 2.81 (m,
52 2H), 2.72 – 2.68 (m, 3H), 2.61 (s, 1H), 2.58 (s, 3H), 2.45 (dd, *J* = 15.5, 10.1 Hz, 0.65H), 2.37 (dt,
53 *J*_t = 9.3 Hz, *J*_d = 4.6 Hz, 0.65H), 2.08 – 2.03 (pent overlapping s, *J*_{pent} = 7.3 Hz, 1.35H), 1.52 (s,
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3 1.85H). ^{13}C NMR (151 MHz, CD_3OD) δ 174.2, 173.5, 171.8, 171.3, 138.0, 137.6, 129.0, 128.9,
4 128.4, 128.0, 126.6, 126.2, 59.7, 59.3, 57.8, 53.1, 49.4, 46.8, 41.1, 41.0, 40.2, 39.0, 36.0, 35.2,
5 35.0, 30.3, 27.8, 27.5, 19.8, 19.1, 17.2. HRMS (HESI-TOF) m/z calc for $(\text{M}+\text{H})^+$ ($\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_3$)⁺
6 291.1709, found 291.1700.
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9 Associated Content

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11 X-ray structures for compounds **7d trans**, **9g trans** and **10**. ^{13}C NMR spectral analysis of
12 piperazine scaffolds for stereochemical determination. ^1H and ^{13}C NMR spectral data for all
13 isolated compounds. Chiral analytical data for all piperazine scaffolds shown in Table 1 as well
14 as for compounds **2a**, **2f**, **13** and **16**. This material is available free of charge via the Internet at
15 <http://pubs.acs.org>.
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25
26 S.K.G.R. is the lead contributor to this work. S.C. is a supporting contributor. I.O.R. is an
27 additional contributor.
28
29

30 Notes

31
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33

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