Synthesis and SAR of Thrombin Inhibitors Incorporating a Novel 4-Amino-Morpholinone Scaffold: Analysis of X-ray Crystal Structure of Enzyme Inhibitor Complex

Jonas W. Nilsson,[†] Ingemar Kvarnström,[†] Djordje Musil,[‡] Ingemar Nilsson,*[,⊥] and Bertil Samulesson*[,§,#]

Department of Chemistry, Linköping University, S-581 83 Linköping, Sweden, AstraZeneca R&D, Structural Chemistry Laboratory, S-481 83 Mölndal, Sweden, Department of Organic Chemistry, Stockholm University, S-106 91 Stockholm, Sweden, Medivir AB, Lunastigen 7, S-141 44 Huddinge, Sweden, and AstraZeneca R&D, Medicinal Chemistry, S-431 83 Mölndal, Sweden

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A 4-amino-2-carboxymethyl-3-morpholinone structural motif derived from malic acid has been used to mimic D-Phe-Pro in the thrombin inhibiting tripeptide D-Phe-Pro-Arg. The arginine in D-Phe-Pro-Arg was replaced by the more rigid P1 truncated *p*-amidinobenzylamine (Pab). These new thrombin inhibitors were used to probe the inhibitor binding site of α-thrombin. The best candidate in this series of thrombin inhibitors exhibits an in vitro IC_{50} of 0.130 μM. Interestingly, the stereochemistry of the 4-amino-2-carboxymethyl-3-morpholinone motif is reversed for the most active compounds compared to that of a previously reported 2-carboxymethyl-3-morpholinone series. The X-ray crystal structure of the lead inhibitor cocrystallized with α-thrombin is discussed.

Introduction

Thromboembolic disorders are the major cause of morbidity and mortality in the developed world. Several acute diseases including deep venous thrombosis, pulmonary embolism, unstable angina, restenosis following angioplasty, and arterial thrombosis are caused by undesired blood clotting events.[1] The clinical syndromes of thromboembolism are triggered by an excessive stimulation of the coagulation cascade.[2] Two crucial steps in the cascade are (i) the formation of thrombin via the prothrombinase complex, consisting of factor Va, Xa, and phospholipids, and (ii) the subsequent proteolytic cleavage of fibrinogen by thrombin, resulting in the generation of the insoluble fibrin clot matrix. Additionally, thrombin mediates platelet activation thereby inducing their adhesion to the fibrin network. Inhibition or activation of enzymes in the coagulation cascade will thus influence blood-clotting events. Not surprisingly, thrombin, as a key player in the cascade, has been the subject of intensive pharmaceutical research, and numerous inhibitors of thrombin have been reported. In addition, substantial effort has also been directed toward FXa inhibition, the final mediator in the formation of thrombin.[3]

Many resources have been put toward identifying small molecule, noncovalently bound, orally active inhibitors for thrombin as well as for FXa.

The active site of thrombin is mainly defined by the specificity (S1) pocket, the hydrophobic proximal (S2) pocket, and the hydrophobic distal (D) pocket. The specificity pocket consists of a hydrophobic "channel" with the carboxylic acid of Asp189 and two backbone carbonyls in the bottom of the pocket, and the latter form strong ionic interactions with amine-, guanidine-, or amidine-type structures located at the terminus of a hydrophobic spacer. The proximal pocket is defined on three sides by the Tyr60A and Trp60D side chains of the 60-insertion loop, the imidazole ring of His57, and the isobutyl group of Leu99 in the enzyme. The larger distal pocket is mainly made up of the side chains of Trp215, Ile174, and Leu99. Other important interactions with potential inhibitors include hydrogen bonding to the β-sheet segment from Ser214-Trp215-Gly216.

Using the classical D-Phe-Pro-Arg motif of thrombin inhibitors and aided by the X-ray crystal structure of the covalently bound inhibitor PPACK[4] in complex with thrombin, a number of drug candidates and late stage investigational drugs such as melagatran (EXANTA)[3,5] have been developed.

Semple et al. have recently reported on the design and synthesis of highly potent thrombin inhibitors incorporating a novel P2-(3-amino-1-carboxymethyl-2-piperidinone) scaffold into the inhibitor CVS 1578 (**1**)[6] (In complex **B**, Figure 1). This motif emanates from opening and reconnecting the proline ring in Bajusz aldehyde (**2**)[7] (In complex **A**, Figure 1). The directional vectors from the sp^2 hybridized N1-atom and the sp^3-hybridized C3-atom of the piperidinone ring fit well into the S2−S3 pocket of thrombin. This provides a similar β-sheet like hydrogen bond network with the backbone carbonyl of Ser214, as well as hydrogen bonds to Gly216, as observed for thrombin inhibitors having a P2-proline residue such as **2**[7] and PPACK.[4]

We recently reported on the synthesis and SAR of a novel 2-carboxymethyl-3-morpholinone scaffold as a P2-group in thrombin inhibitors[8] (**C**). This resulted in

* Corresponding authors: I. Nilsson tel.: +46-31-776-1361; fax: +46-31-776-3839; e-mail: ingemar.nilsson@astrazeneca.com. B. Samuelsson tel.: +46-8-608-3104; fax: +46-8-608-3199; e-mail: bertil.samuelsson@medivir.se.
[†] Linköping University.
[‡] Structural Chemistry, AstraZeneca R&D.
[§] Stockholm University.
[#] Medivir AB.
[⊥] Medicinal Chemistry, AstraZeneca R&D.

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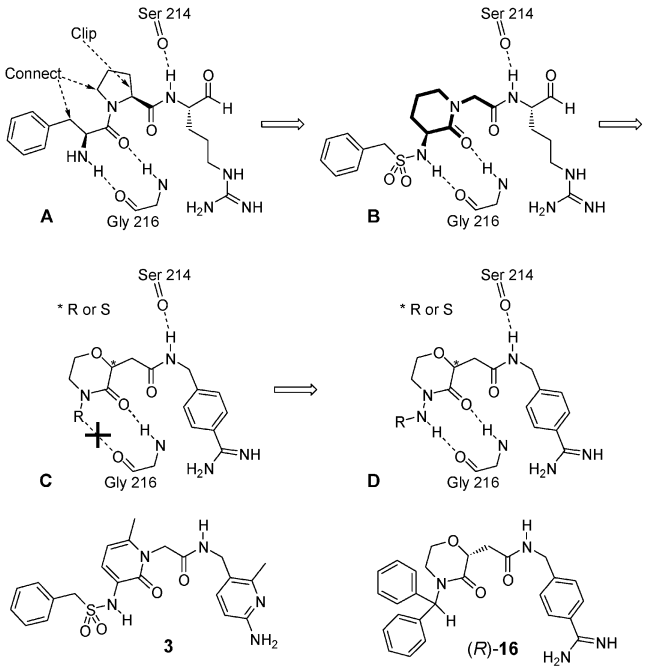


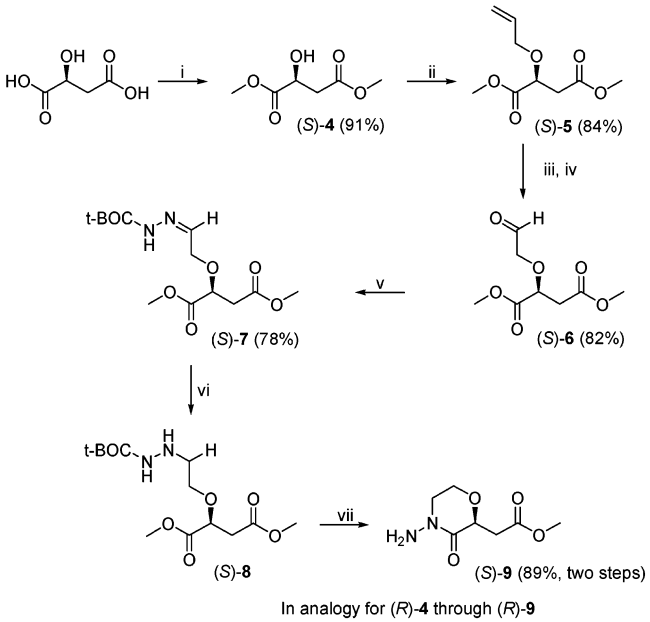
Figure 1. (Upper trace) Schematic drawing describing the development from Bajusz aldehyde[7] (**2**) to CNS 1578[6] (**1**) and interactions with Ser214 and Gly216; (middle trace) The tentative recovery of the interactions to Gly216 in the current series compared to those previously reported;[8] (lower trace) compounds L-374,087[10] (**3**) and (*R*)-**16**.[8]

inhibitors with modest potency. The best inhibitor with (*R*)-diphenylmethyl ((*R*)-**16**, Figure 1) in this series of thrombin inhibitors exhibits an in vitro IC_{50} of 0.724 μM. X-ray studies revealed that the overall fit of the inhibitor to the thrombin active site, extending from the S1-pocket into the S2-pocket and the distal (**D**)-pocket, was quite good and similar to that reported by Semple et al. for **1**,[9] despite the fact that **1** binds covalently through its aldehyde to Ser195. The absence of such a strong interaction may seem devastating, but it has been shown in numerous reports that noncovalently bound thrombin inhibitors can exhibit nanomolar or even subnanomolar affinity as reported by Sanderson et al., who have developed inhibitors based on the 3-amino-2-pyridone-acetamide template. One substance, L-374,087[10] (**3**, Figure 1), is closely related to **1** and binds similarly.

The inhibitor (*R*)-**16**, however, essentially lacks interactions with Gly216, and we argued that the absence of the hydrogen bond donating capability to the C═O of Gly216 could possibly explain the modest activity of these series of compounds. Therefore, we embarked on the synthesis of thrombin inhibitors **D** having the novel 4-amino-2-carboxymethyl-3-morpholinone scaffold. Structural motifs based on **D** are readily available from (*R*)- or (*S*)-malic acid using a synthetic route adapted from that previously reported.[8]

In this report, we describe the synthesis of these compounds having *p*-amidinobenzylamine in the P1 position and mainly lipophilic amines in the P3 position. The lead inhibitor in this series exhibits an in vitro IC_{50} of 0.130 μM. Interestingly, but not unexpected as revealed from docking studies, the more potent compounds have the (*S*)-stereochemistry in the morpholinone moiety in contrast to that observed for the series

Scheme 1. Preparation of Key Intermediates (*S*)-**9** and (*R*)-**9**[a]



[a] Reagents: (i) $SOCl_2$, MeOH; (ii) allyl bromide, silver(I) oxide, toluene; (iii) osmium (VIII) oxide, *N*-methyl morpholine-*N*-oxide, THF/H_2O 3:1; (iv) sodium periodate, THF/H_2O 3:1; (v) hydrazine carboxylic acid *tert*-butyl ester, toluene, 65 °C; (vi) H_2/Pd−C, THF; (vii) reflux H_2O.

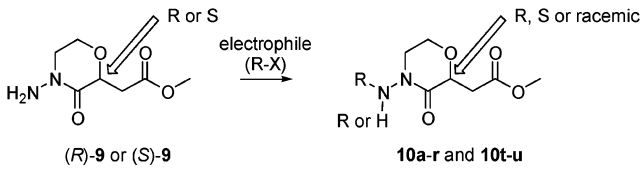
previously reported.[8] Subsequent X-ray studies revealed some unexpected interesting features that are discussed.

Results and Discussion

Synthesis of the Key Intermediate Compounds (*S*)-9 and (*R*)-9. The natural (*S*)-malic acid and the unnatural (*R*)-malic acid are both commercially available and were used as starting materials. Esterification of (*S*)-malic acid was achieved using thionyl chloride in methanol to yield the dimethyl ester (*S*)-**4** in 91% yield (Scheme 1).[11] Alkylation employing allyl bromide and freshly precipitated silver(I) oxide in toluene afforded (*S*)-**5** in 84% yield together with 7% of the bis allylated byproduct (2-allyloxy-succinic acid 1-allyl ester 4-methyl ester). The allyl moiety of (*S*)-**5** was converted into a diol, by treatment with catalytic osmium (VIII) oxide and *N*-methyl morpholine-*N*-oxide. Subsequent oxidative cleavage using sodium periodate yielded the aldehyde (*S*)-**6** in 82% total yield. Heating (*S*)-**6** with hydrazine carboxylic acid *tert*-butyl ester (BOC-hydrazine) in toluene furnished the hydrazone (*S*)-**7** in 78% yield. Catalytic hydrogenation, Pd on carbon, in tetrahydrofuran[11] followed by in situ lactamization and deprotection by refluxing in water resulted in the key intermediate compound (*S*)-**9** in 89% yield. Compound (*R*)-**9** was synthesized using the same procedure starting from (*R*)-malic acid. Enantiomeric purities were >98% ee for both (*S*)-**9** and (*R*)-**9**.[12]

Synthesis of 10a−c. Palladium catalyzed arylaminations is a valuable tool for the synthesis of aniline type structures.[13] Both choice of phosphine ligand and base greatly influence reaction yields and selectivity.[14] Commonly used bases include sodium *tert*-butoxide and cesium carbonate. In this study, use of sodium *tert*-butoxide resulted in complete degradation of starting material, whereas cesium carbonate gave clean reac-

Scheme 2. Derivatization at Nitrogen



tions. From the numerous phosphine ligands described in the literature, we selected the bidentate ligand 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene[15] (XANTPHOS). Thus, (*S*)-**9** was heated at 95 °C for 19 h in toluene containing palladium (II) acetate (10 mol %), XANTPHOS (20 mol %), and the appropriate arylbromide (Scheme 2, Table 1). Compound **10a** was isolated in 33% yield after treatment with 1 equiv of 3-bromonitrobenzene, and compounds **10b** and **10c** were both isolated after reaction with 3 equiv of bromobenzene in 58 and 19% yield, respectively. Not surprisingly, however, compounds **10a**−**c** had racemized under the reaction conditions.

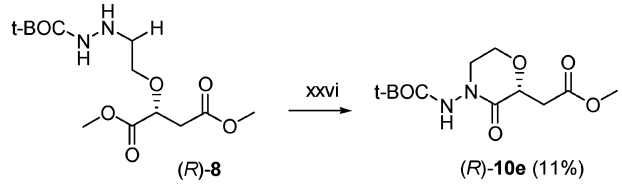
Synthesis of 10d, 10f−**j, 10p**−**r, and 10t.** Reaction of (*R*)-**9** or (*S*)-**9** with various electrophiles (Table 1), including acid chlorides, sulfonyl chlorides, chloroformate, and isocyanate, afforded the corresponding amides (**10d**, **10g**), sulfonamides (**10f**, **10h, 10p**−**r, 10t**), carbamate (**10j**), and urea (**10i**) in good yield (67−100%) with the exception of amide (*R*)-**10g** (49% yield) and sulfonamide (*S*)-**10h** (39%).[16]

Synthesis of 10k−**n and 10u.** The hydrazones formed by reacting the appropriate aldehyde with hydrazide (*R*)-**9** or (*S*)-**9** were stable and could be readily isolated after column chromatography. Thus, using a two-step reductive alkylation procedure polyalkylations could be avoided. Phenylacetaldehyde was reacted with (*R*)-**9** in toluene at 60 °C (Table 1) overnight to form the corresponding hydrazone. Catalytic hydrogenation in THF for 2 h afforded (*R*)-**10k** in quantitative yield. Using a similar procedure with 3-phenylpropionaldehyde afforded both (*R*)-**10*l*** (55%) and (*R*)-**10m** (37%). Other reductive alkylations afforded (*S*)-**10k**, (*S*)-**10*l***, (*R*)-**10n**, (*S*)-**10n**, and (*S*)-**10u** in varying yields (47−85%). It should be noted that for (*R*)-**10n** and (*S*)-**10n** the low yields are due to partial reductive loss of the benzyl group. For example, in the synthesis of (*S*)-**10n**, where 27% of the desired product is formed together with 52% of the reduced product, identical to hydrazide (*S*)-**9**.

Since preliminary results showed that (*R*)-**9** did not form the expected product upon reaction with di-*tert*-butyl dicarbonate, [(*R*)-4-*tert*-butoxycarbonylamino-3-oxo-morpholin-2-yl]-acetic acid methyl ester ((*R*)-**10e**) was synthesized by performing the lactamization of the BOC-hydrazino derivative (*R*)-**8** in refluxing toluene (Scheme 3) instead of water (6 days, only partial reaction) delivering (*R*)-**10e** in low yield (11%). No improvement in reaction time or yield was observed when base (triethylamine) was added.

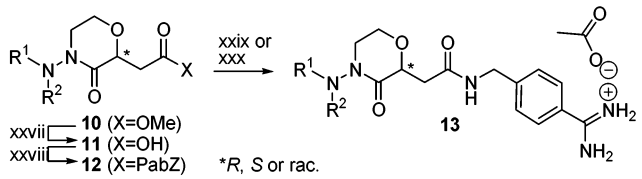
Incorporation of the P1 Moiety. Synthesis of 13a−**u.** Ester hydrolysis of **10a**−**u** using 3 equiv of lithium hydroxide in water−methanol (2:3) (Scheme 4) was followed by coupling with 4-benzyloxycarbonylamidino-benzylamine dihydrochloride (PabZ × 2HCl) using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA) in dimethylformamide (DMF). After deprotection of the Z-group by hydrogenation[17] or treatment with trifluoromethanesulfonic acid and purification using preparative HPLC, **13a**−**u** were isolated as acetic acid salts (Table 2).

Scheme 3. Alternative Lactam Formation Reaction[a]



[a] Reagents: (xxvi) reflux, toluene, 6 days.

Scheme 4. Incorporation of the P1 Moiety[a]



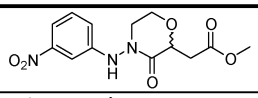
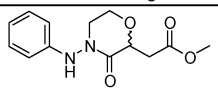
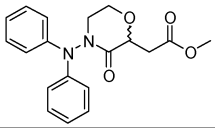
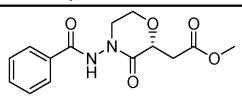
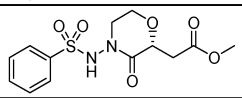
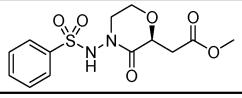
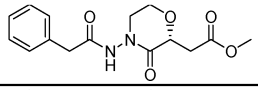
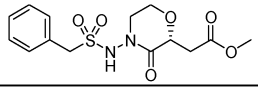
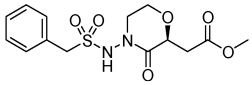
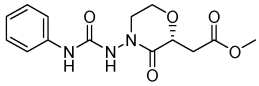
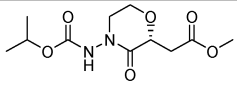
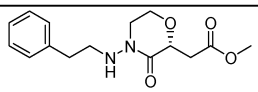
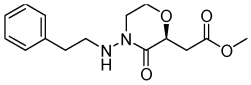
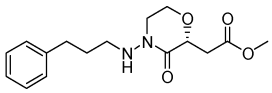
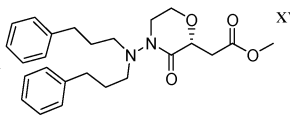
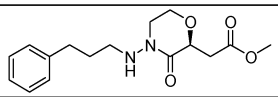
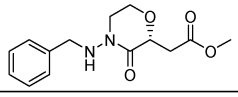
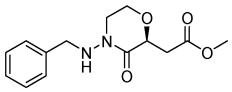
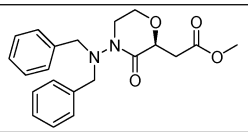
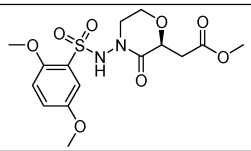
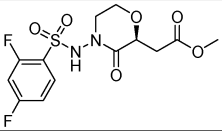
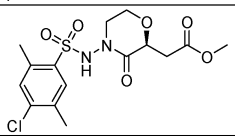
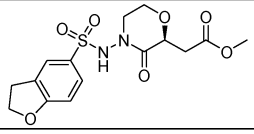
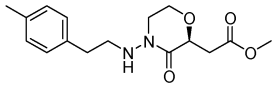
[a] Reagents: (xxvii) 3 equiv of LiOH in MeOH−H_2O (2:3); (xxviii) PabZ × 2HCl; HATU; DIPEA; DMF; (xxix) H_2/Pd−C, preparative HPLC in MeOH−H_2O−AcOH; (xxx) HSO_3CF_3; anisole; DCM, preparative HPLC in MeOH−H_2O−AcOH.

Structure−**Activity Relationship.** Initially, synthetic efforts were focused on compounds having the (*R*)-stereochemistry with respect to the 4-amino-2-carboxymethyl-3-morpholinone moiety ((*R*)-**13a** to (*R*)-**13m**), as this was the preferred stereochemistry in the reported 2-carboxymethyl-3-morpholinone series.[8] To our disappointment, most of these compounds with comparable length of the P3 side chain showed very modest potency, and in no case was inhibition significantly improved compared to similar compounds in previously reported series (Table 3).

However, docking studies using the Glide and MacroModel modeling software,[18] performed with the X-ray of thrombin from the complex with either **1** or (*R*)-**16** (Figure 1), strongly indicated that the structures with the (*S*)-configuration of the morpholinone fitted at least as well as the (*R*)-form to the thrombin active site. Synthesis of some (*S*)-morpholinone analogues were consequently undertaken, and much to our satisfaction this resulted in admittedly modest but significantly improved potency toward thrombin compared to the corresponding (*R*)-morpholinones. Compounds (*S*)-**13f** (IC_{50} 2.89 μM), (*S*)-**13h** (9.15 μM), (*S*)-**13k** (1.79 μM), and (*S*)-**13l** (4.74 μM) exhibited between 4 and 20 times higher activity compared to that of (*R*)-**13f** (37.7 μM), (*R*)-**13h** (36.8 μM), (*R*)-**13k** (35.6 μM), and (*R*)-**13l** (45.4 μM).

On the basis of these promising results, a small set of compounds was designed to evaluate if further improvements in potency could be obtained. A virtual library of 141 structures, from a set of 41 aldehydes and 100 benzenesulfonyl chlorides as P3-group precursors in combination with P1-*p*-amidinobenzylamine and P2-(*S*)-4-amino-2-carboxymethyl-3-morpholinone, was enumerated. On the basis of the results from the Glide docking studies of this virtual library to the X-ray of **1**, a set of six compounds were chosen to be synthesized

Table 1. Preparation of Compounds **10a**−**d**, **10f**−**r**, and **10t**−**u**

product	react. cond.[a]	isolated yields
10a 	viii	33%
10b 		58%
10c 	ix	19%
(*R*)-**10d** 	x	97 %
(*R*)-**10f** 	xi	quant
(*S*)-**10f** 	xi	85%
(*R*)-**10g** 	xii	49%
(*R*)-**10h** 	xiii	86%
(*S*)-**10h** 	xiii	39%
(*R*)-**10i** 	xiv	quant
(*R*)-**10j** 	xv	93%
(*R*)-**10k** 	xvi, xvii	quant
(*S*)-**10k** 	xvi, xvii	63%
(*R*)-**10l** 		55%
(*R*)-**10m** 	xviii, xvii	37%
(*S*)-**10l** 	xviii, xvii	85%
(*R*)-**10n** 	xix, xvii	50%
(*S*)-**10n** 	xix, xvii	56%[b]
(*S*)-**10o** 	xx	60%
(*S*)-**10p** 	xxi	82%
(*S*)-**10q** 	xxii	67%
(*S*)-**10r** 	xxiii	89%
(*S*)-**10t** 	xxiv	98%
(*S*)-**10u** 	xxv, xvii	47%

[a] Reagents: (viii) *m*-bromonitrobenzene (1 equiv), Pd(II) acetate, XANTPHOS, toluene, 95 °C; (ix) PhBr (3 equiv), Pd(II) acetate, XANTPHOS, toluene, 95 °C; (x) BzCl, pyridine; (xi) $PhSO_2Cl$, pyridine; (xii) BnCOCl, pyridine; (xiii) $BnSO_2Cl$, pyridine; (xiv) PhNCO, toluene; (xv) ^{i}PrOCOCl, pyridine; (xvi) BnCOH, toluene; (xvii) H_2/Pd−C, THF; (xviii) $Ph(CH_2)_2COH$, toluene; (xix) PhCHO, $PhCH(OH)_2$, toluene; (xx) BnBr, DIPEA, $NaHCO_3$, LiI, DMF; (xxi) 2,5-$(MeO)_2PhSO_2Cl$, pyridine; (xxii) 2,4-F_2PhSO_2Cl, pyridine; (xxiii) 4-Cl-2,5-Me_2PhSO_2Cl, pyridine; (xxiv) 3,4-(-CH_2CH_2O-)$PhSO_2Cl$, pyridine; (xxv) *p*-tol-CH_2CHO, toluene. [b] 52% of starting material was regenerated after synthesis. Yield corresponds to consumed starting material.

((*S*)-**13p** to (*S*)-**13u**), together with compounds (*R*)-**13n** and (*S*)-**13n**, which were selected for comparison with the corresponding P3-phenylethyl analogues of the previously reported 2-carboxymethyl-3-morpholinone series. Gratifyingly, we were able to improve potency by more than 1 order of magnitude, and the 2,5-dimethoxybenzenesulfonyl analogue (*S*)-**13p** (0.130 μM) showed the highest potency. The trend for the benzenesulfonyl analogues is clearly that the lipophilic substition ((*S*)-**13p**, (*S*)-**13r**, (*S*)-**13s**) is well accommodated by the distal (D)-pocket as indicated by the docking studies, whereas the *N*-(2,4-di-fluoro benzenesulfonyl) analogue (*S*)-**13q** (8.14 μM), not surprisingly, creates less favorable polar interactions with the lipophilic D-pocket and results in slightly decreased potency compared to the unsubstituted analogue (*S*)-**13f** (2.89 μM). Similarly the P3-*N*-(2-*p*-tolyl-ethyl) analogue (*S*)-**13u** (0.961 μM) results in a modest improvement of potency compared to the P3-*N*-(2-phenylethyl) analogue (*S*)-**13k** (1.79 μM). The two P3-(*R*)- and (*S*)-benzyl derivatives ((*R*)-**13n**, (*S*)-**13n)** both exhibit lower potency but may be the only compounds with almost the same

Table 2. Yields in % for the Different Derivatives

step(s)	a	b	c	d	e	f	f	g	h	h	i	j	k
10−**13**	42	59	71	42	39	29	48	53	50	21	74	94	30
config of **13**	rac	rac	rac	R	R	R	S	R	R	S	R	R	R

step(s)	k	l	l	m	n	n	o	p	q	s	r	t	u
10−**13**	81	89	15	74	100	100	53	60	55	27[a]	15[a]	60	68
config of **13**	S	R	S	R	R	S	S	S	S	S	S	S	S

[a] Both (*S*)-**13r** and (*S*)-**13s** were formed from (*S*)-**12r** in the reaction. Therefore, a total yield of 100% is the theoretical maximum.

Table 3. Thrombin IC_{50} Values

name	P3 substituent(s)	thrombin IC_{50} (μM)
13a	3-amino-phenyl	>32
13b	phenyl	34.4
13c	diphenyl	4.88
(*R*)-**13d**	benzoyl	106
(*R*)-**13e**	*tert*-butoxycarbonyl	54.7
(*R*)-**13f**	benzenesulfonyl	37.7
(*S*)-**13f**	benzenesulfonyl	2.89
(*R*)-**13g**	phenylacetyl	>133
(*R*)-**13h**	phenylmethanesulfonyl	36.8
(*S*)-**13h**	phenylmethanesulfonyl	9.15
(*R*)-**13i**	*N*-anilinocarbonyl	150
(*R*)-**13j**	isopropoxycarbonyl	80.6
(*R*)-**13k**	phenethyl	35.6
(*S*)-**13k**	phenethyl	1.79
(*R*)-**13l**	3-phenyl-propyl	45.4
(*S*)-**13l**	3-phenyl-propyl	4.74
(*R*)-**13m**	bis-(3-phenyl-propyl)	36.2
(*R*)-**13n**	benzyl	13.6
(*S*)-**13n**	benzyl	10.6
(*S*)-**13o**	dibenzyl	19.8
(*S*)-**13p**	2,5-dimethoxybenzenesulfonyl	0.130
(*S*)-**13q**	2,4-difluorobenzenesulfonyl	8.14
(*S*)-**13r**	4-chloro-2,5-dimethylbenzenesulfonyl	0.164
(*S*)-**13s**	2,5-dimethylbenzenesulfonyl	0.247
(*S*)-**13t**	2,3-dihydro-benzofuran-5-sulfonyl	0.486
(*S*)-**13u**	2-*p*-tolyl-ethylamino	0.961

Table 4. Parameters and Statistics for X-ray Crystallography Data Collection and Refinement

description	data
no of measurements	398 756
no of unique reflections	29 307
data completeness (%)	97.0
R_{merge}[a]	0.058
no of atoms in refined model	2588
protein	2239
cofactor (hirugen)	90
inhibitor	35
solvent	324
resolution range in refinement (Å)	35−1.85
r.m.s. deviation for bond length (Å)	0.006
angles (°)	1.39
R_{cryst}[b]	0.226
R_{free}	0.251

[a] $R_{merge} = S_h S_i(|I(h,i) - \langle I(h)\rangle|)/S_h S_i I(h,i)$ where $I(h,i)$ is the intensity value of the ith measurement of h, and $\langle I(h)\rangle$ is the corresponding mean value of h for all i measurements of h. [b] $R_{cryst} = S_{hkl}(|F_o - F_c|)/S_{hkl}|F_o|$. $|F_o|$ and $|F_c|$ are observed and calculated structure factor amplitudes, respectively.

potency (13.6 and 10.6 μM, respectively). The racemic analogues **13a**−**c** may also fall into this group.

To provide additional information regarding the binding in the thrombin active site of this series of compounds, the most potent compound (*S*)-**13p** was cocrystallized in complex with α-thrombin and subjected to X-ray analysis. Figure 2 shows the schematic picture

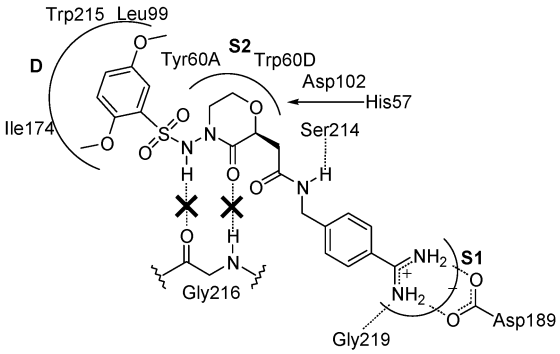


Figure 2. The thrombin nomenclature and the important interactions between (*S*)-**13p** and thrombin are schematically outlined.

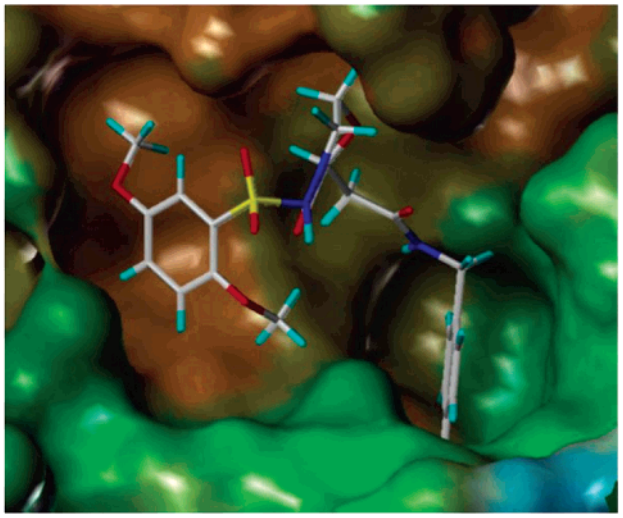


Figure 3. The Connolly surface map of the X-ray structure of the α-thrombin-(*S*)-**13p** (white) complex at 1.85 Å resolution.

of the important interactions between the thrombin inhibitor (*S*)-**13p** and the active site of thrombin. The X-ray structure of the α-thrombin−(*S*)-**13p** complex (Figure 3) shows that the expected S1−S2−D-pocket binding of (*S*)-**13p** is observed, and the overall alignment of (*S*)-**13p** compares well with that of **1** (Figure 4), but some interesting differences are also observed. The strongly basic amidine of the P1-*p*-amidinobenzyl group forms a salt bridge with Asp189 with N−O distances of 2.70 and 2.74 Å, similar to that of the guanidine group of **1**. The N−H of the P1−P2 amide linkage forms a weak hydrogen bond to C=O of Ser214, of almost the same length as for **1** (N−O distances 3.15, 3.01 Å, respectively). The 3-morpholinone ring occupies the S2-pocket, but moves deeper than the piperidone of **1** into the S2-pocket. This results in a significant movement of Tyr60A and Trp60D of the 60-insertion loop as can be seen in the alignment of the X-ray structures in Figure 5. As a consequence of this deeper penetration, there are no hydrogen bond interactions with Gly216 with N−O distances of 5.1 Å between NH-Gly216 and the C=O of the 3-morpholinone and 6.1 Å between C=O of Gly216 and the NH of the P2−P3 amide linkage.

The 2,5-dimethoxy-benzenesulfonyl group fits very nicely into the lipophilic D-pocket, where the phenyl ring is positioned much the same as the P2-phenyl group of **1** and with the 5-methoxy group in the small cavity

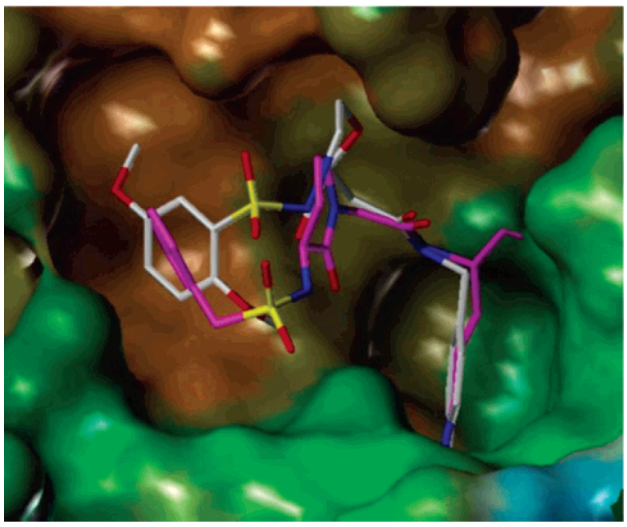


Figure 4. The superimposition of the α-thrombin-(*S*)-**13p** (white) and α-thrombin-**1** (magenta) X-ray crystal structures.

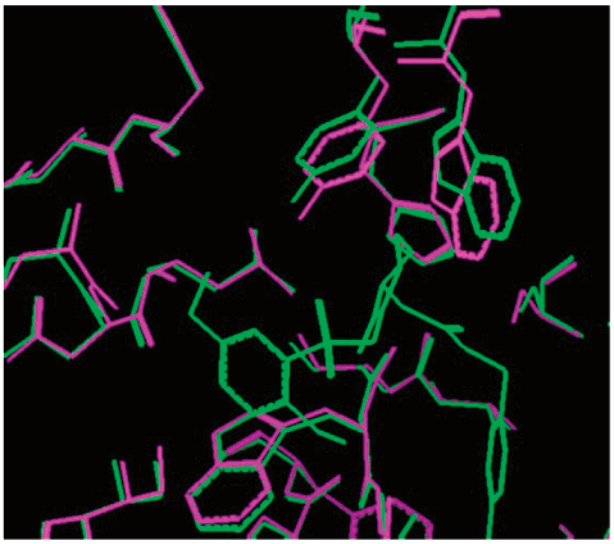


Figure 5. The alignment of the X-ray crystal structures of α-thrombin-(*S*)-**13p** (green) including ligand and α-thrombin-**1** (magenta).

between Leu99 and Tyr60A. The 2-methoxy makes quite close contact with the C═O of Gly216 (C−O distance 3.3 Å) and should due to seemingly less favorable electrostatic interactions not contribute to binding, but may have a stabilizing effect of the binding conformation of the benzenesulfonyl moiety. The comparisons with the compound series reported by Semple et al.[9] as well as with the series of Sanderson et al.[10] are quite interesting. In their series, the P3-benzylsulfonamides are more potent than the P3-phenylsulfonamides, whereas the reverse is observed in our series. This fits nicely with the differences in the location of the corresponding P2-group. The hydrogen bond network with Gly216 observed in their series results in a position and directional vector of the P2-NH−P3-SO_2 linkage that allows the benzyl group to fit smoothly in the D-pocket, whereas the same properties of the P2-NH−P3-SO_2 linkage in our series gives less room for the larger benzyl group compared to the phenyl group resulting in the reversed order of binding. Obviously, our initial

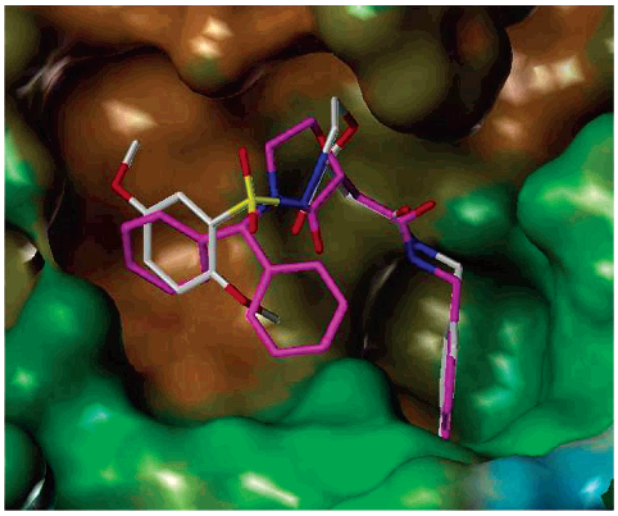


Figure 6. The superimposition of the α-thrombin-(*S*)-**13p** (white) and α-thrombin-(*R*)-**16** (magenta) X-ray crystal structures.

thought that the introduction of a hydrogen bond donating capability going from **C** to **D** (Figure 1) would result in the reestablishment of this hydrogen bond network was not correct.

To understand the propensity of the NH to form hydrogen bonds, the Cambridge Crystallographic Databank[19] was searched for intermolecular N−O distances between C═O and NH of the generic sequences C−S(O_2)−NH−N−C═O and C−S(O_2)−NH−O−R, but this did not result in any hits. Therefore, NH of C−S(O_2)−NH−C was examined. For the 82 records found, the N−O bond distance, not unexpectedly, displayed an almost Gaussian distribution between 2.7 and 3.1 Å, and one would, in line with expectation, assume that the hydrogen bond donating capability would be in the same range for the NH of the C−S(O_2)−NH−N−C═O motif. However, it may well be that the pK_a of the NH is such that it is prone to deprotonation. A search in the Advanced Chemistry Development pK_a database[20] revealed that a similar compound *N*′-acetyl-*N*′-methylbenzenesulfonohydrazide had a pK_a of 7.85. When ionized, this would result in an unfavorable interaction between a negatively charged nitrogen and the electronegative C═O of Gly216, that is relieved by movement of the 3-morpholinone deep into the S2-pocket. In line with this, the X-ray of (*R*)-**16**-α-thrombin, which lacks the possibility of deprotonation, has a location of the P2-group that less deeply penetrates the S2-pocket compared to that of (*S*)-**13p** (Figure 6) and does not result in a movement of Tyr60A and Trp 60D compared to **1**. Admittedly, this may also be a consequence of the larger constraints of the more rigid P3-diphenyl group of (*R*)-**16**. A similar penetration of the S2-pocket would result in a substantial decrease of favorable hydrophobic interaction between one of the phenyl groups of (*R*)-**16** and the D-pocket. Anyhow, the stereochemistry of the more potent compounds are reversed in the current series compared to the (*R*)-**16** analogues, and we see no other reasons for these observations. In line with the proposed deprotonation of the P3-phenylsulfonyl derivatives, the P3-*N*-(2-phenylethyl) (*S*)-**13k**, (*S*)-**13u**, and P3−*N*-benzyl (*S*)-**13n** analogues have far less acidic C−NH−N−C═O groups and should not result in un-

favorable interactions with the C=O of Gly216 and tentatively results in closer contact between these two groups. This seems to be in agreement with the experimental observations. The larger phenylethyl-group in (*S*)-**13k** and (*S*)-**13u** now seems to be accommodated in the D-pocket, and this results in higher potency than the P3-*N*-benzyl analogue (*S*)-**13n**. The reversed potencies are observed for the corresponding benzylsulfonyl and phenylsulfonyl analogues, (*S*)-**13h** and (*S*)-**13f**, respectively. The presence of a favorable interaction for the N−H is supported by the decrease in activity of the *N*,*N*-dibenzyl analogue (*S*)-**13o** compared to the N-benzyl analogue (*S*)-**13n**.

Finally, it must be mentioned that the directional vector of the sp^3 hybridized 2-carbon as well as the sp^3 hybridization of the 1-oxygen and 5,6-carbons of the 3-morpholinone ring in this and our previous series of compounds may have a negative influence on the thrombin affinity compared to what has been observed for inhibitors based on the 3-amino-2-pyridone-acetamide template.[10] Furthermore, in that series of compounds the subnanomolar potency is partly obtained through the methyl substituent on the 6-carbon, corresponding to the 1-oxygen of the 3-morpholinone ring, that provide an additional hydrophobic interaction in the S2-pocket and secondly acts as a conformational constraint for the N-acetamide group.

Conclusions

Thrombin inhibitors with respectable potency have been prepared from the novel 4-amino-2-carboxymethyl-3-morpholinone template. The best inhibition of thrombin was achieved by (*S*)-**13p**, with an IC_{50} value of 0.130 μM. In this series of compounds, as predicted by modeling studies, the stereochemistry of the 4-amino-2-carboxymethyl-3-morpholinone motif is reversed for the most active compounds compared to that of the previously reported 2-carboxymethyl-3-morpholinone series. The potency, as observed in the X-ray crystal structure of (*S*)-**13p**, is obtained without utilizing the well-documented hydrogen bond network from the P2−P3 linkage to Gly 216. A possible rationale for this is a deprotonation of the SO_2−NH−N−CO moiety, which results in a repulsion from the C=O of Gly 216 and explains the deeper penetration of the P2-group into the S2-pocket and also results in a closer contact of the P3-group with the interior of the D-pocket and thereby favoring smaller P3-groups. This provides an explanation for the reversed potency trend observed for the different P3-groups for this series of compounds compared to the compound series reported by Semple et al.[9] as well as with the series of Sanderson et al.[10] In the latter two, P3-benzylsulfonamides are more potent than P3-phenylsulfonamides, whereas the opposite is true in our series. In the series of Semple et al. and Sanderson et al., the interaction of the sulfonamide linkage with Gly216 creates room for slightly larger P3-groups, whereas this is not the case in our series.

Experimental Section

Thrombin Inhibition Measurements. The thrombin inhibitor potency was measured with a chromogenic substrate method in a Plato 3300 robotic microplate processor (Rosys AG, CH-8634 Hombrechtikon, Switzerland), using 96-well, half volume microtiter plates (Costar, Cambridge, MA, USA; Cat No 3690). Stock solutions of test substance in DMSO (72 μL), 10 mmol/L, alternatively 1 mmol/L were diluted serially 1:3 (24 + 48 μL) with DMSO to obtain 10 different concentrations, which were analyzed as samples in the assay, together with controls and blanks. As a control sample, melagatran was analyzed. The dilutions of each test substance were analyzed consecutively, row-wise on the microtiter plate, with wash-cycles between substances to avoid cross-contamination. First 2 μL of test sample or DMSO for the blank were added, followed by 124 μL of assay buffer (0.05 mol/L Tris-HCl pH 7.4 at 37 °C, ionic strength 0.15 adjusted with NaCl, bovine serum albumin, ICN Biomedicals, Inc, USA, 1 g/L) and 12 μL of chromogenic substrate solution (S-2366, Chromogenix, Mölndal, Sweden) and finally 12 μL of α-thrombin solution (human α-thrombin, Haematologic Technologies Inc., Essec Junction, Vermont, USA), in buffer, was added, and the samples were mixed. The final assay concentrations were test substance 0.0068−133 μmol/L, respectively, 0.00068−13.3 μmol/L, S-2366 0.30 mmol/L and α-thrombin 0.14 nmol/L. The linear absorbance increase at 405 nm during a 40 min incubation at 37 °C was used for calculation of percent inhibition for the test samples, as compared to blanks without inhibitor. The IC_{50} value, corresponding to the inhibitor concentration that caused 50% inhibition of the thrombin activity, was calculated by fitting the data to a three parameter equation by Microsoft XLfit.

X-ray Crystallography. Human α-thrombin was purchased from Enzyme Research Laboratories, Inc., South Bend, IN, and hirugen from American Diagnostica, Inc., Greenwich, CT. Hirugen−thrombin complex was prepared according to the method of Skrzypczak-Jankun et al.[21] The crystallization was done as described previously.[22] The X-ray diffraction data were collected on a MAR−II imaging plate system, MAR Research, Hamburg, Germany, using Cu Kα radiation from a rotating anode. The data were reduced and scaled using DENZO and SCALEPACK[23] programs. The hirugen−α-thrombin structure previously examined in our laboratory was used in the refinement of the (*R*)-**16**−α-thrombin complex structure. The refinement was performed using REFMAC (CCP4 package)[24] with subsequent runs of CNX.[25] Statistics for X-ray data collection and refinement are presented in Table 1.

General. TLC analysis was performed on Merck precoated 60 F_{254} plates. Column chromatography was performed using silica gel 60 (0.040−0.063 mm, Merck). Organic phases were dried over magnesium sulfate monohydrate. Concentrations were performed by rotary evaporation. NMR-spectra were recorded on a Varian Mercury 300 MHz instrument and Varian Iova 600 MHz instrument using chloroform-*d* or methanol-d_4 as solvent.[26] Purification was performed on a semi-preparative Gynkotek P580/UVD1705 HPLC system equipped with an external low-pressure exponential gradient mixer. High-resolution mass spectrometry (HRMS) data were obtained in positive ion mode using a double focusing Finnigan MAT900S equipped with electrospray interface. Analytical HPLC experiments for selected compounds were performed on a Varian Prostar 330 Photodiode Array detector equipped with a Varian Prostar 230 ternary gradient solvent delivery system and a Chromasil C8 column (10 μm, 250 × 4.6 mm). Analysises were performed with two distinct solvent systems. System A: gradient of 10−90% acetonitrile over 12 min @ 1 mL/min. System B: gradient of 10−90% 2-propanol over 12 min @ 1 mL/min.[27] To all solvents were added trifluoroacetic acid (0.1% v/v). Peaks were detected at 220−250 nm. Consecutive analysis of 100 μL injections containing 0, 0.25, 1.0, and 4.0 μg of the analytes were performed to deduce background signals.

(*S*)-(2-Oxy-ethoxy)-succinic Acid Dimethyl Ester ((*S*)-6). Synthesis of (*S*)-**6** is described in a previous publication.[8]

(*S*)-[2-(*tert*-Butoxycarbonyl-hydrazono)-ethoxy]-succinic Acid Dimethyl ester ((*S*)-7). (*S*)-(2-Oxy-ethoxy)-succinic acid dimethyl ester ((*S*)-**3**) (0.083 g, 0.40 mmol) and hydrazine carboxylic acid *tert*-butyl ester (0.053 g, 0.40 mmol) was dissolved in toluene (9 mL) and heated to 65 °C overnight. Concentration afforded (*S*)-**7** (0.124 g, 97%) as a white solid of acceptable purity. $[\alpha]_D^{25}$ −33.2° (*c* 1.1 $CHCl_3$); ^{1}H NMR ($CDCl_3$,

600 MHz) δ 1.48 (s, 9H), 2.74 (dd, J = 16.5; 8.3 Hz, 1H), 2.80 (dd, J = 16.5; 4.2 Hz, 1H), 3.69 (s, 3H), 3.75 (s, 3H), 4.20−4.25 (m, 1H), 4.32−4.37 (m, 2H), 7.18−7.24 (broad, 1H), 7.96−8.06 (broad, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 28.4, 37.8, 52.3, 52.6, 70.3, 74.5, 142.4, 170.6, 171.8; HRMS *m/z* calcd for $C_{13}H_{23}N_2O_7^+$ (MH^+): 319.1505. Found: 319.1508.

(*S*)-2-[2-(*N*′-*tert*-Butoxycarbonyl-hydrazino)-ethoxy]-succinic Acid Dimethyl Ester ((*S*)-8). (*S*)-**7** (0.169 g, 0.528 mmol) was stirred under hydrogen gas atmosphere in THF (10 mL) containing Pd (37 mg, 10% on activated carbon) for 18 h. The palladium was filtered off, and (*S*)-**8** (0.171 g, 100%) was isolated as a colorless oil after concentration. $[\alpha]_D^{25}$ −48.5° (*c* 1.06 $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 9H), 2.65−2.85 (m, 2H), 2.91−3.06 (m, 2H), 3.51−3.61 (m, 1H), 3.68 (s, 3H), 3.74 (s, 3H), 3.71−3.81 (m, 1H), 4.26−4.35 (m, 1H), 6.28−6.42 (broad, 1H); ^{13}C NMR ($CDCl_3$, 150.8 MHz) δ 28.5, 37.8, 51.1, 52.3, 52.5, 68.9, 69.3, 75.5, 156.7, 170.9, 172.2.

[(*S*)-4-Amino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-9). (*S*)-**8** (0.171 g, 0.528 mmol) and water (50 mL) was heated at 60 °C for 7 h. After concentration column chromatography was performed using DCM/methanol 15:1 as eluent. Pure (*S*)-**9** (0.076 g, 77%) was isolated as a white solid. $[\alpha]_D^{25}$ −91.7° (*c* 1.01 $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.75 (dd, J = 16.4; 7.1 Hz, 1H), 2.89 (dd, J = 16.4; 4.0 Hz, 1H), 3.29−3.35 (m, 1H), 3.62 (s, 3H), 3.65−3.75 (m, 1H), 3.75−3.85 (m, 1H), 3.92−4.00 (m, 1H), 4.40−4.45 (m, 1H), 4.44 (s, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.0, 50.6, 52.0, 63.4, 74.6, 167.6, 171.1; HRMS *m/z* calcd for $C_7H_{13}N_2O_4^+$ (MH^+): 189.0875. Found: 189.0880.

(*R*)-4 to (*R*)-9. Synthesis was performed according to the methods of (*S*)-**4** to (*S*)-**9**. Analytical data:

(*R*)-[2-(*tert*-Butoxycarbonyl-hydrazono)-ethoxy]-succinic Acid Dimethyl Ester ((*R*)-7). $[\alpha]_D^{25}$ +33.8° (*c* 1.05 $CHCl_3$); HRMS *m/z* calcd for $C_{13}H_{23}N_2O_7^+$ (MH^+): 319.1505. Found: 319.1509.

(*R*)-2-[2-(*N*′-*tert*-Butoxycarbonyl-hydrazino)-ethoxy]-succinic Acid Dimethyl Ester ((*R*)-8). $[\alpha]_D^{25}$ +48.4° (*c* 1.05 $CHCl_3$).

[(*R*)-4-Amino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-9). $[\alpha]_D^{25}$ +97.6° (*c* 0.77 $CHCl_3$); HRMS *m/z* calcd for $C_7H_{13}N_2O_4^+$ (MH^+): 189.0875. Found: 189.0882.

Racemic [4-(3-Nitro-phenylamino)-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester (10a). A Schlenk tube equipped with a water aspirator and argon supply was charged with toluene (0.7 mL), palladium(II) acetate (9.2 mg, 0.041 mmol) and 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene (XANTPHOS, 0.048 g, 0.082 mmol).

A solution of (*S*)-**9** (0.077 g, 0.41 mmol) in toluene (2.9 mL) was added followed by *m*-bromonitrobenzene (0.049 mL, 0.41 mmol). The Schlenk tube was gently evacuated with the water aspirator without letting the solution boil and then filled with argon. The evacuation/argon filling procedure was repeated 5 times, after which cesium carbonate (0.268 g, 0.82 mmol) was added. The mixture was heated to 95 °C and stirred under argon for 19 h. The solution was cooled to room temperature and subjected to column chromatography using DCM/methanol 15:1 as eluent. Fractions containing the arylated product together with some residual XANTPHOS was pooled concentrated and subjected anew to column chromatography, this time using toluene/ethyl acetate 1:1 as eluent. Pure **10a** (0.042 g, 33%) was isolated. No residual rotation of polarized light was detected. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.94 (dd, J = 16.8; 4.4 Hz, 1H), 3.01 (dd, J = 16.8; 5.5 Hz, 1H), 3.47−3.60 (m, 1H), 3.73 (s, 3H), 3.99−4.24 (m, 3H), 4.58 (dd, J = 5.5; 4.4 Hz, 1H), 6.94 (s, 1H), 7.12 (ddd, J = 8.1; 2.3; 1.0 Hz, 1H), 7.37 (dd, J = 8.1; 8.1 Hz, 1H), 7.12 (dd, J = 2.2; 2.3 Hz, 1H), 7.12 (ddd, J = 8.1; 2.2; 1.0 Hz, 1H).

Racemic (3-Oxo-4-phenylamino-morpholin-2-yl)-acetic Acid Methyl Ester (10b) and Racemic (4-Diphenylamino-3-oxo-morpholin-2-yl)-acetic Acid Methyl Ester (10c). See procedure for **10a**. Instead of 1 equiv of *m*-bromonitrobenzene, 3 equiv of bromobenzene was used. Pure **10b** (0.063 g, 58%) and **10c** (0.027 g, 19%) was isolated. No residual rotation of polarized light was detected. **10b** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.92 (dd, J = 16.5; 6.4 Hz, 1H), 3.00 (dd, J = 16.4; 4.4 Hz, 1H), 3.44−3.55 (m, 1H), 3.71 (s, 3H), 3.89−4.17 (m, 3H), 4.59 (dd, J = 6.4; 4.4 Hz, 1H), 6.72 (s, 1H), 6.78−6.83 (m, 2H), 6.90−6.96 (m, 1H), 7.21−7.28 (m, 2H). **10c** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.94 (dd, J = 16.5; 6.5 Hz, 1H), 3.02 (dd, J = 16.5; 4.3 Hz, 1H), 3.51−3.57 (m, 1H), 3.69 (s, 3H), 3.93−4.20 (m, 3H), 4.61 (dd, J = 6.5; 4.3 Hz, 1H), 7.00−7.08 (m, 2H), 7.09−7.20 (m, 4H), 7.26−7.34 (m, 4H).

[(*R*)-4-Benzoylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10d). (*R*)-**9** (0.026 g, 0.136 mmol) was dissolved in pyridine (4 mL). Benzoyl chloride (0.032 mL, 0.272 mmol) was added, and the solution was stirred for 25 min after which ice (1 g) was added. Concentration was followed by column chromatography using toluene/ethyl acetate (1:3) as eluent to yield (*R*)-**10d** (0.038 g, 97%). $[\alpha]_D^{25}$ +80.3° (*c* 0.77 $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.98 (dd, J = 16.5; 4.7 Hz, 1H), 3.05 (dd, J = 16.5; 6.1 Hz, 1H), 3.56−3.63 (m, 1H), 3.71 (s, 3H), 3.82−3.93 (m, 1H), 4.02−4.16 (m, 2H), 4.59 (dd, J = 6.1; 4.7 Hz, 1H), 7.20−7.30 (m, 2H), 7.32−7.41 (m, 1H), 7.69−7.66 (m, 2H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.9, 50.8, 52.3, 63.7, 75.0, 127.7, 128.6, 132.4, 133.5, 169.1, 171.2.

[(*R*)-4-*tert*-Butoxycarbonylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10e). (*R*)-**8** (0.038 g, 0.118 mmol) was refluxed in toluene (60 mL) for 3 days. Concentration followed by column chromatography using toluene/ethyl acetate 1:1 as eluent gave (*R*)-**10e** (0.004 g, 11%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.48 (s, 9H), 2.87 (dd, J = 16.5; 6.8 Hz, 1H), 2.98 (dd, J = 16.5; 4.1 Hz, 1H), 3.47−3.57 (m, 1H), 3.71 (s, 3H), 3.84−4.14 (m, 3H), 4.57 (dd, J = 6.8; 4.1 Hz, 1H), 6.68 (broad s, 1H).

[(*R*)-4-Benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10f). (*R*)-**9** (0.022 g, 0.118 mmol) was dissolved in pyridine (5 mL). Benzenesulfonyl chloride (0.018 mL, 0.142 mmol) was added, and the solution was stirred for 20 h. The solvent was removed in vacuo and the pyridine residues were removed by coevaporation with toluene (3 times). The crude product was dissolved in ethyl acetate and the solution was washed 2 times with water. The combined aqueous extracts were extracted with chloroform and the combined organic extracts were concentrated to give (*R*)-**10f** (0.040 g, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.54 (dd, J = 16.7; 6.9 Hz, 1H), 2.63 (dd, J = 16.7; 4.0 Hz, 1H), 3.64 (s, 3H), 3.81−4.12 (m, 4H), 4.24 (dd, J = 6.9; 4.0 Hz, 1H), 7.48−7.56 (m, 2H), 7.60−7.67 (m, 1H), 7.86 (broad s, 1H), 7.87−7.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.6, 51.6, 52.2, 63.7, 74.6, 128.8, 129.2, 134.3, 136.7, 167.6, 170.6.

[(*S*)-4-Benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10f). Compound (*S*)-**10f** was prepared in 85% yield from (*S*)-**9** according to the method for preparation of (*R*)-**10f** and showed equivalent analytical data.

[(*R*)-3-Oxo-4-phenylacetamido-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10g). (*R*)-**9** (0.019 g, 0.101 mmol) was dissolved in pyridine (5 mL). Phenylacetyl chloride (0.016 mL, 0.121 mmol) was added, and the solution was stirred for 22 h. Since almost no reaction could be observed, additional phenylacetyl chloride (0.053 mL, 0.404 mmol) was added, and stirring was continued for 7 more hours. After concentration, the residue was taken up in DCM (10 mL) and washed with water (100 mL). The aqueous phase was then extracted 2 times with DCM (10 mL). The combined organic phases were concentrated. After column chromatography using toluene/ethyl acetate 1:1, (*R*)-**10g** (0.015 g, 49%) was isolated. Low yield should be explained by observed polymerization of phenylacetyl chloride. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.92 (d, J = 5.4 Hz, 2H), 3.44−3.52 (m, 1H), 3.62 (s, 2H), 3.68 (s, 3H), 3.75−3.87 (m, 1H), 3.97−4.05 (m, 2H), 4.55 (t, J = 5.4 Hz, 1H), 7.24−7.38 (m, 5H).

[(*R*)-3-Oxo-4-phenylmethanesulfonylamino-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10h). (*R*)-**9** (0.019 g, 0.101 mmol) was dissolved in pyridine (5 mL). Phenylmethanesulfonyl chloride (0.023 g, 0.121 mmol) was added, and the solution was stirred for 22 h. More phenylmethanesulfonyl chloride (0.023 g, 0.121 mmol) was added and stirring

continued for 2 more hours. Concentration was followed by column chromatography using toluene/ethyl acetate 1:1. Pure (*R*)-**10h** (0.030 g, 86%) was isolated. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.91 (dd, J = 16.8; 4.2 Hz, 1H), 3.04 (dd, J = 16.8; 5.0 Hz, 1H), 3.62−3.69 (m, 1H), 3.72 (s, 3H), 3.85 (ddd, J = 13.1; 12.2; 3.0 Hz, 1H), 3.99−4.10 (m, 2H), 4.34 (d, J = 13.7 Hz, 1H), 4.45 (dd, J = 5.0; 4.2 Hz, 1H), 4.56 (d, J = 13.7 Hz, 1H), 7.34−7.42 (m, 3H), 7.45−7.52 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.8, 52.2, 52.3, 59.2, 64.0, 74.8, 128.1, 129.0, 129.1, 131.2, 168.8, 170.8.

[(*S*)-3-Oxo-4-phenylmethanesulfonylamino-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10h). (*S*)-**9** (0.041 g, 0.221 mmol) was dissolved in pyridine (10 mL). Phenylmethanesulfonyl chloride (0.0843 g, 0.442 mmol) was added, and the solution was stirred for 24 h. Since TLC showed no reaction, the temperature was raised to 35 °C and more phenylmethanesulfonyl chloride (0.253 g, 1.33 mmol) was added over a period of 3 days. Concentration was followed by washing (chloroform/water). Column chromatography using toluene/ethyl acetate 1:1 afforded pure (*S*)-**10h** (0.029 g, 39%).[16] Analytical data was equivalent with that for (*R*)-**10h**.

[(*R*)-3-Oxo-4-(3-phenyl-ureido)-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10i). (*R*)-**9** (0.019 g, 0.101 mmol) was dissolved in toluene (10 mL). Phenylisocyanate (0.013 mL, 0.121 mmol) was added, and the solution was stirred for 22 h. More phenylisocyanate (0.044 g, 0.404 mmol) was added and stirring continued for 3 more hours. After concentration, residual phenylisocyanate was scavenged by heating in methanol (20 mL, 40 °C) for 5 min. Concentration followed by column chromatography using DCM/methanol 15:1 gave pure (*R*)-**10i** (0.031 g, 100%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.90 (dd, J = 16.9; 4.3 Hz, 1H), 3.11 (dd, J = 16.9; 4.7 Hz, 1H), 3.43−3.48 (m, 1H), 3.73 (s, 3H), 3.90−4.04 (m, 3H), 4.46 (app. t, J = 4.5 Hz, 1H), 6.98 (ddt, J = 7.8; 7.0; 1.2 Hz, 1H), 7.16−7.24 (m, 2H), 7.42−7.48 (m, 1H), 7.86 (broad s, 1H), 8.18 (broad s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.0, 51.7, 52.5, 63.7, 74.7, 119.9, 123.4, 128.9, 138.6, 155.2, 169.7, 171.9.

[(*R*)-4-Isopropoxycarbonylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10j). (*R*)-**9** (0.019 g, 0.101 mmol) was dissolved in pyridine (5 mL) and cooled with an ice bath. Isopropyl chloroformate (0.121 mL, 1 M solution in toluene, 0.121 mmol) was added, and the solution was stirred for 4 h. After concentration and coevaporation with toluene three times, the residue was chromatographized using toluene/ethyl acetate 1:1 as eluent. Insoluble pyridinium chloride was left on top of the column. Pure (*R*)-**10j** (0.026 g, 93%) was isolated. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (d, J = 6.3 Hz, 6H), 2.86 (dd, J = 16.5; 7.0 Hz, 1H), 2.96 (dd, J = 16.5; 4.1 Hz, 1H), 3.46−3.53 (m, 1H), 3.69 (s, 3H), 3.85−4.10 (m, 3H), 4.56 (dd, J = 7.0; 4.1 Hz, 1H), 4.96 (sept., J = 6.3 Hz, 1H), 7.01 (broad s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 22.1, 37.0, 51.3, 52.2, 63.7, 70.5, 75.1, 155.8, 168.5, 171.0.

[(*R*)-3-Oxo-4-phenethylamino-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10k). To a solution of (*R*)-**9** (0.021 g, 0.113 mmol) in toluene (10 mL), phenylacetaldehyde (0.016 mL, 0.136 mmol) was added. After heating overnight at 60 °C, solvents were removed in vacuo. Analysis of the hydrazone showed ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.90 (dd, J = 16.5; 7.2 Hz, 1H), 3.06 (dd, J = 16.5; 3.8 Hz, 1H), 3.47−3.54 (m, 1H), 3.69 (s, 3H), 3.70−3.85 (m, 3H), 3.91 (ddd, J = 12.1; 10.7; 3.3 Hz, 1H), 4.15 (ddd, J = 12.1; 4.5; 2.0 Hz, 1H), 4.60 (dd, J = 7.2; 3.8 Hz, 1H), 7.13−7.37 (m, 5H), 7.67 (t, J = 5.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.4, 39.9, 47.0, 52.2, 63.1, 75.4, 127.2, 128.3, 129.1, 129.3, 136.3, 151.8, 171.1. The crude hydrazone was dissolved in tetrahydrofuran (8 mL), palladium (11 mg, 10% on activated carbon) was added and stirring in hydrogen gas atmosphere was performed for 2 h. Filtration was followed by concentration to give crude (*R*)-**10k** (0.033 g, 100%) that was used without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.82 (t, J = 7.3 Hz, 2H), 2.84 (dd, J = 16.4; 6.8 Hz, 1H), 2.96 (dd, J = 16.4; 4.1 Hz, 1H), 3.12−3.20 (m, 2H), 3.29−3.35 (m, 1H), 3.69 (s, 3H), 3.70−3.79 (m, 2H), 3.97−4.04 (m, 1H), 4.45 (dd, J = 6.8; 4.1 Hz, 1H), 7.16−7.34 (m, 5H).

[(*S*)-3-Oxo-4-phenethylamino-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10k). Compound (*S*)-**10k** was prepared from (*S*)-**9** according to the method for preparation of (*R*)-**10k.** Purification using column chromatography, toluene/ethyl acetate 1:1 gave pure (*S*)-**10k** (0.021 g, 63% two steps from (*S*)-**9**). Analytical data were equivalent with that of (*R*)-**10k**.

[(*R*)-3-Oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10*l*) and {(*R*)-4-[Bis-(3-phenyl-propyl)-amino]-3-oxo-morpholin-2-yl}-acetic Acid Methyl Ester ((*R*)-10m). To a solution of (*R*)-**9** (0.019 g, 0.101 mmol) in toluene (10 mL), 3-phenylpropanal (0.017 mL, 0.131 mmol) was added. After heating for 24 h at 70 °C, solvents were removed in vacuo. The residue was dissolved in THF (8 mL) and palladium (8.5 mg, 10% on active carbon) was added. After stirring of the sample in hydrogen gas atmosphere for 70 min, the palladium was filtered off and the supernatant was concentrated. Column chromatography using toluene/ethyl acetate (1:1) gave pure (*R*)-**10*l*** (0.017 g, 55%) and (*R*)-**10m** (0.016 g, 37%). (*R*)-**10*l*** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.76−1.87 (m, 2H), 2.66−2.73 (m, 2H), 2.85 (dd, J = 16.4; 6.8 Hz, 1H), 2.83−2.97 (broad, 2H), 2.97 (dd, J = 16.4; 4.2 Hz, 1H), 3.30−3.37 (m, 1H), 3.70 (s, 3H), 3.69−3.79 (m, 1H), 3.79−3.89 (m, 1H), 3.99−4.07 (m, 1H), 4.49 (dd, J = 6.8; 4.2 Hz, 1H), 7.14−7.22 (m, 3H), 7.24−7.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 29.8, 33.4, 37.0, 48.8, 48.9, 52.1, 63.6, 74.7, 126.1, 128.61, 128.63, 167.3, 171.1. (*R*)-**10m** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.68−1.92 (broad m, 4H), 2.56−2.76 (broad, 4H), 2.76−2.90 (broad, 2H), 2.87 (dd, J = 16.4; 6.3 Hz, 1H), 2.95 (dd, J = 16.4; 4.3 Hz, 1H), 3.18−3.33 (broad m, 3H), 3.67 (s, 3H), 3.61−3.80 (m, 2H), 3.94−4.01 (m, 1H), 4.39 (dd, J = 6.3; 4.3 Hz, 1H), 7.13−7.23 (m, 6H), 7.24−7.31 (m, 4H).

[(*S*)-3-Oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10*l*). To a 5 mL toluene solution of (*S*)-**9** (0.019 g, 0.101 mmol), 3-phenylpropanal (0.027 mL, 0.202 mmol) was added. After heating of the sample for 5 h at 70 °C, solvents were removed in vacuo and the formed hydrazone was purified using column chromatography with toluene/ethyl acetate 1:1 as eluent. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.79−1.90 (m, 2H), 2.69−2.79 (m, 2H), 2.89 (dd, J = 16.5; 7.2 Hz, 1H), 3.05 (dd, J = 16.5; 3.9 Hz, 1H), 3.42−3.51 (m, 1H), 3.69 (s, 3H), 3.68−3.79 (m, 1H), 3.91 (ddd, J = 3.3; 10.8; 12.1 Hz, 1H), 4.15 (ddd, J = 2.0; 4.5; 12.1 Hz, 1H), 4.59 (dd, J = 7.2; 3.9 Hz, 1H), 7.16−7.33 (m, 5H), 7.67 (t, J = 5.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 32.9, 34.9, 37.4, 47.1, 52.1, 63.1, 75.3, 126.5, 128.6, 128.8, 140.8, 153.6, 171.1. The hydrazone was then dissolved in THF (6 mL). Palladium (10% on activated carbon, 5 mg) was added, and the solution was stirred under hydrogen gas atmosphere for 3 h. Filtration and evaporation yielded the amine (*S*)-**10*l*** (0.031 g, quant.). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.76−1.89 (m, 2H), 2.64−2.74 (m, 2H), 2.79−3.01 (m, 4H), 3.31−3.38 (m, 1H), 3.69 (s, 3H), 3.65−3.90 (m, 2H), 3.99−4.07 (m, 1H), 4.49 (dd, J = 6.8; 4.2 Hz, 1H), 7.14−7.32 (m, 5H).

[(*R*)-4-Benzylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*R*)-10n). To a 8 mL toluene solution of (*R*)-**9** (0.0139 g, 0.074 mmol) was added benzaldehyde dimethylacetal (0.0275 mL, 0.185 mmol) and benzaldehyde (0.414 mL, 4.07 mmol). The solution was heated to 80 °C for 2 days. The solvents were removed in vacuo and excess benzaldehyde was removed using high vacuum (oil pump). Column chromatography was performed using toluene/ethyl acetate 1:1 as eluent to achieve pure hydrazone (0.0198 g, 97%). The hydrazone was dissolved in THF (6 mL). Palladium (10% on activated carbon, 7 mg) was added, and the solution was stirred under hydrogen gas atmosphere for 5 min. Filtration and evaporation were followed by column chromatography using toluene/ethyl acetate 1:1 as eluent to achieve pure amine (*R*)-**10n** (0.011 g, 52%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.84 (dd, J = 16.3; 7.0 Hz, 1H), 2.97 (dd, J = 16.3; 4.1 Hz, 1H), 3.15−3.23 (m, 1H), 3.57−3.68 (m, 1H), 3.71 (s, 3H), 3.74 (ddd, J = 3.1; 10.6; 11.6 Hz, 1H), 3.93 (ddd, J = 2.0; 4.1; 11.6 Hz, 1H), 4.02 (s, 2H), 4.49 (dd, J = 7.0; 4.1 Hz, 1H), 7.26−7.42 (m, 5H); ^{13}C NMR ($CDCl_3$, 75.5

MHz) δ 37.0, 49.6, 52.1, 54.0, 63.6, 74.7, 128.0, 128.7, 129.4, 137.3, 171.1, 175.6.

[(*S*)-4-Benzylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10n). Compound (*S*)-**10n** was prepared from (*S*)-**9** according to the method for preparation of (*R*)-**10n** and showed equivalent analytical data. The hydrazone was however hydrogenolyzed for 10 min instead of 5, which explains the lower yield of amine (*S*)-**10n** (27%). Also 52% of starting material (*S*)-**9** was regenerated. Yield of (*S*)-**10n** relative to consumed (*S*)-**9** was therefore 56%.

[(*S*)-4-Dibenzylamino-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10o). (*S*)-**9** (0.025 g, 0.136 mmol), was dissolved in DMF (10 mL). DIPEA (0.118 mL, 0.285 mmol), sodium hydrogencarbonate (0.342 g, 4.07 mmol), LiI (0.004 g, 0.030 mmol), and benzylbromide (0.114 mL, 0.952 mmol) was added and the suspension was heated to 50 °C for 7 h after which solvents was removed in vacuo. Column chromatography using DCM/methanol 15:1 as eluent afforded pure (*S*)-**10o** (0.0326 g, 60%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.52−2.64 (m, 1H), 2.66 (dd, *J* = 16.2; 7.9 Hz, 1H), 2.97 (dd, *J* = 16.2; 3.8 Hz, 1H), 2.98−3.09 (m, 2H), 3.36−3.48 (m, 1H), 3.69 (s, 3H), 4.02 (d, *J* = 12.0 Hz, 1H), 4.21 (d, *J* = 12.6 Hz, 1H), 4.29 (dd, *J* = 7.9; 3.8 Hz, 1H), 4.51 (d, *J* = 12.6 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 7.24−7.44 (m, 10H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.0, 52.1, 52.8, 57.6, 58.4, 63.2, 75.0, 127.8, 128.48, 128.52, 129.7, 129.9, 137.9, 138.1, 168.8, 171.3.

[(*S*)-4-(2,5-dimethoxy-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10p). (*S*)-**9** (0.015 g, 0.080 mmol) was dissolved in pyridine (4 mL). 2,5-Dimethoxybenzenesulfonyl chloride (0.0227 g, 0.096 mmol) was added, and the solution was stirred for 24 h. The solvent was removed in vacuo and the pyridine residues were removed by coevaporation with toluene (3 times). The crude product was purified using column chromatography with toluene/ethyl acetate 1:1 as eluent to achieve (*S*)-**10p** (0.0253 g, 82%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.61 (dd, *J* = 16.6; 6.5 Hz, 1H), 2.68 (dd, *J* = 16.6; 4.1 Hz, 1H), 3.64 (s, 3H), 3.80 (s, 3H), 3.79−3.92 (m, 2H), 3.94−4.06 (m, 2H), 4.00 (s, 3H), 4.27 (dd, *J* = 6.5; 4.1 Hz, 1H), 6.96 (d, *J* = 9.0 Hz, 1H), 7.10 (dd, *J* = 9.0; 3.1 Hz, 1H), 7.37 (d, *J* = 3.1 z, 1H), 8.46 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.7, 52.1, 52.3, 56.3, 57.4, 63.7, 74.5, 114.1, 115.3, 121.7, 126.3, 152.1, 152.9, 167.4, 170.6.

[(*S*)-4-(2,4-Difluoro-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10q). See procedure for (*S*)-**10p**. 2,4-Difluorobenzenesulfonyl chloride (0.0203 g, 0.096 mmol) was used as electrophile. Yield of (*S*)-**10q** was (0.0194 g, 67%).^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.69 (d, *J* = 5.0 Hz, 2H), 3.65 (s, 3H), 3.83−3.95 (m, 2H), 3.95−4.10 (m, 2H), 4.27 (t, *J* = 5.0 Hz, 1H), 6.92−7.02 (m, 2H), 7.84−7.93 (m, 1H), 7.99 (broad s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.6, 52.1, 52.2, 63.8, 74.5, 106.1 (t, *J* = 25.6 Hz), 111.8 (dd, *J* = 22.0; 3.7 Hz), 133.0 (d, *J* = 10.6 Hz), 170.5. Some signals lost in the noise (approximately 124 (dd), 161 (dd), 167 (dd)).

[(*S*)-4-(4-Chloro-2,5-dimethyl-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10r). See procedure for (*S*)-**10p**. 4-Chloro-2,5-dimethylbenzenesulfonyl chloride (0.0229 g, 0.096 mmol) was used as electrophile. Yield of (*S*)-**10r** was (0.0277 g, 89%).^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H), 2.59 (dd, *J* = 15.8; 6.1 Hz, 1H), 2.66 (dd, *J* = 15.8; 4.3 Hz, 1H), 2.68 (s, 3H), 3.65 (s, 3H), 3.78−3.90 (m, 2H), 3.94−4.09 (m, 2H), 4.24 (dd, *J* = 6.1; 4.3 Hz, 1H), 7.30 (s, 1H), 7.74 (s, 1H), 7.80 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 19.7, 20.4, 36.6, 51.8, 52.2, 63.7, 74.5, 133.1, 133.3, 133.5, 135.0, 138.1, 138.3, 170.5.

[(*S*)-4-(2,3-Dihydro-benzofuran-5-sulfonylamino)-3-oxo-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10t). (*S*)-**9** (0.025 g, 0.133 mmol) was dissolved in pyridine (7 mL). 2,3-Dihydro-1-benzofuran-5-sulfonyl chloride (0.0401 g, 0.183 mmol) was added and the solution was stirred for 90 min. The solvent was removed in vacuo and the crude product was purified using column chromatography with toluene/ethyl acetate 1:1 as eluent to achieve (*S*)-**10t** (0.0484 g, 98%).^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.57 (dd, *J* = 16.6; 7.1 Hz, 1H), 2.67 (dd, *J* = 16.6; 4.1 Hz, 1H), 3.28 (t, *J* = 8.9 Hz, 2H), 3.64 (s, 3H), 3.80−4.10 (m, 4H), 4.29 (dd, *J* = 7.1; 4.1 Hz, 1H), 4.68 (t, *J* = 8.9 Hz, 2H), 6.80−6.84 (m, 1H), 7.63−7.71 (m, 2H), 7.78 (broad s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 29.1, 36.7, 51.4, 52.2, 63.7, 72.7, 74.6, 109.6, 126.0, 127.8, 128.7, 130.6, 165.3, 167.4, 170.6.

[(*S*)-3-Oxo-4-(2-*p*-tolyl-ethylamino)-morpholin-2-yl]-acetic Acid Methyl Ester ((*S*)-10u). To a solution of (*S*)-**9** (0.0206 g, 0.110 mmol) in toluene (10 mL), *p*-tolylacetaldehyde (0.0176 g, 0.131 mmol) was added. After heating overnight at 75 °C, solvents were removed in vacuo. The crude hydrazone was dissolved in tetrahydrofuran (8 mL), palladium (11 mg, 10% on activated carbon) was added and the solution was stirred in hydrogen gas atmosphere for 24 h. Filtration was followed by concentration and column chromatography using toluene/ethyl acetate 1:1 as eluent to give pure (*S*)-**10u** (0.0159 g, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.31 (s, 3H), 2.78 (t, *J* = 7.3 Hz, 2H), 2.84 (dd, *J* = 16.4; 6.8 Hz, 1H), 2.96 (dd, *J* = 16.4; 4.1 Hz, 1H), 3.10−3.18 (m, 2H), 3.31−3.37 (m, 1H), 3.70 (s, 3H), 3.67−3.84 (m, 2H), 3.98−4.06 (m, 1H), 4.46 (dd, *J* = 6.8; 4.1 Hz, 1H), 7.07−7.15 (m, 4H);^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.2, 34.2, 37.0, 48.8, 50.3, 52.1, 63.6, 74.7, 128.7, 129.4, 136.1, 141.7, 171.1.

Substituted [4-Amino-3-oxo-morpholin-2-yl]-acetic Acid (11). General Procedure. Hydrolysis was performed by stirring **10** with 3 equiv of lithium hydroxide in methanol/water 3:2 (100 mL per mmol of **10**) until no residual starting material was shown by TLC (DCM/methanol 15:1). Hydrochloric acid (1 M, 3.3 equiv) was added, and solvents were removed in vacuo to give products **11** as white solids. The crude carboxylic acid was used without further purification.

Racemic [4-(3-Nitro-phenylamino)-3-oxo-morpholin-2-yl]-acetic Acid (11a). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.85 (dd, *J* = 16.7; 4.3 Hz, 1H), 2.93 (dd, *J* = 16.7; 5.6 Hz, 1H), 3.45−3.53 (m, 1H), 3.92−4.19 (m, 3H), 4.56 (dd, *J* = 5.6; 4.3 Hz, 1H), 7.22 (ddd, *J* = 8.1; 2.3; 1.0 Hz, 1H), 7.42 (td, *J* = 8.1; 0.4 Hz, 1H), 7.61 (td, *J* = 2.3; 0.4 Hz, 1H), 7.66 (ddd, *J* = 8.1; 2.3; 1.0 Hz, 1H).

Racemic (3-Oxo-4-phenylamino-morpholin-2-yl)-acetic Acid (11b). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.88 (d, *J* = 5.3 Hz, 2H), 3.41−3.48 (m, 1H), 3.85−3.95 (m, 1H), 3.98−4.08 (m, 1H), 4.12 (ddd, *J* = 11.9; 4.7; 2.0 Hz, 1H), 4.55 (t, *J* = 5.3 Hz, 1H), 6.79−6.87 (m, 3H), 7.16−7.24 (m, 2H).

[(*R*)-4-Benzoylamino-3-oxo-morpholin-2-yl]-acetic Acid ((*R*)-11d). ^{1}H NMR (methanol-d_4/D_2O (2:1), 300 MHz) δ 2.82−3.02 (m, 2H), 3.52−3.62 (m, 1H), 3.90−4.02 (m, 1H), 4.05−4.21 (m, 2H), 4.67 (dd, *J* = 6.7; 4.5 Hz, 1H), 7.49−7.57 (m, 2H), 7.59−7.67 (m, 1H), 7.59−7.67 (m, 2H); ^{13}C NMR (methanol-d_4/D_2O (2:1), 75.5 MHz) δ 36.7, 50.8, 63.2, 74.9, 127.6, 128.9, 131.5, 133.0, 169.5, 173.6.

[(*R*)-4-*tert*-Butoxycarbonylamino-3-oxo-morpholin-2-yl]-acetic Acid ((*R*)-11e). ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.47 (s, 9H), 2.62−2.96 (m, 2H), 3.37−3.46 (m, 1H), 3.75−3.87 (m, 1H), 3.87−4.01 (m, 1H), 4.05 (ddd, *J* = 12.0; 4.6; 2.2 Hz, 1H), 4.50−4.57 (m, 1H).

[(*R*)-4-Benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetic Acid ((*R*)-11f). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.44 (dd, *J* = 15.4; 6.9 Hz, 1H), 2.51 (dd, *J* = 15.4; 4.2 Hz, 1H), 3.66−3.73 (m, 1H), 3.83−4.07 (m, 3H), 4.23−4.29 (m, 1H), 7.51−7.58 (m, 2H), 7.61−7.68 (m, 1H), 7.61−7.68 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 37.8, 52.1, 63.1, 75.5, 128.3, 128.9, 133.5, 138.4, 168.6, 174.3.

[(*S*)-4-Benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetic Acid ((*S*)-11f). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.61−2.73 (m, 2H), 3.78−4.14 (m, 4H), 4.20−4.30 (m, 1H), 7.46−7.58 (m, 2H), 7.59−7.68 (m, 1H), 7.86−7.95 (m, 2H), 8.37 (broad s, 1H), 9.16 (v. broad s, 1H).

[(*R*)-3-Oxo-4-phenylacetamido-morpholin-2-yl]-acetic Acid ((*R*)-11g). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.72 (dd, *J* = 16.2; 7.5 Hz, 1H), 2.84 (dd, *J* = 16.2; 3.8 Hz, 1H), 3.34−3.41 (m, 1H), 3.59 (s, 2H), 3.74−3.85 (m, 1H), 3.91−4.08 (m, 2H), 4.55 (dd, *J* = 7.5; 3.8 Hz, 1H), 7.20−7.36 (m, 5H).

[(*R*)-3-Oxo-4-phenylmethanesulfonylamino-morpholin-2-yl]-acetic Acid ((*R*)-11h). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.83 (dd, *J* = 16.6; 4.1 Hz, 1H), 2.95 (dd, *J* = 16.7; 5.4 Hz,

1H), 3.53−3.61 (m, 1H), 3.86−4.07 (m, 3H), 4.40 (d, J = 13.7 Hz, 1H), 4.51 (dd, J = 5.4; 4.1 Hz, 1H), 4.56 (d, J = 13.7 Hz, 1H), 7.31−7.38 (m, 3H), 7.43−7.50 (m, 2H).

[(*R*)-3-Oxo-4-(3-phenyl-ureido)-morpholin-2-yl]-acetic Acid ((*R*)-11i). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.83 (dd, J = 16.6; 4.0 Hz, 1H), 2.92 (dd, J = 16.6; 5.7 Hz, 1H), 3.41−3.53 (m, 1H), 3.90−4.11 (m, 3H), 4.46−4.52 (m, 1H), 6.98−7.06 (m, 1H), 7.21−7.29 (m, 2H), 7.21−7.29 (m, 1H).

[(*R*)-4-Isopropoxycarbonylamino-3-oxo-morpholin-2-yl]-acetic Acid ((*R*)-11j). ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.26 (d, J = 6.2 Hz, 6H), 2.71 (dd, J = 16.2; 7.6 Hz, 1H), 2.86 (dd, J = 16.2; 3.8 Hz, 1H), 3.39−3.47 (m, 1H), 3.77−3.88 (m, 1H), 3.89−4.02 (m, 1H), 4.07 (ddd, J = 11.9; 4.5; 2.1 Hz, 1H), 4.54 (dd, J = 7.6; 3.8 Hz, 1H), 4.91 (sept. J = 6.2 Hz, 1H).

[(*R*)-3-Oxo-4-phenethylamino-morpholin-2-yl]-acetic Acid ((*R*)-11k). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.66−2.88 (m, 4H), 3.08−3.15 (m, 2H), 3.32−3.41 (m, 1H), 3.63−3.85 (m, 2H), 3.94−4.04 (m, 1H), 4.39 (dd, J = 6.2; 4.3 Hz, 1H), 7.11−7.31 (m, 5H).

[(*R*)-3-Oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetic Acid ((*R*)-11l). ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.73−1.84 (m, 2H), 2.64−2.72 (m, 2H), 2.77−2.82 (m, 2H), 2.84−2.92 (m, 2H), 3.34−3.41 (m, 1H), 3.66−3.76 (m, 1H), 3.84 (ddd, J = 11.9; 10.4; 3.0 Hz, 1H), 4.02 (ddd, J = 11.9; 4.2; 2.0 Hz, 1H), 4.41−4.45 (m, 1H), 7.10−7.29 (m, 5H).

[(*S*)-3-Oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetic Acid ((*S*)-11l). ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.91−2.06 (m, 2H), 2.63−3.08 (m, 6H), 3.20−3.30 (m, 1H), 3.59−3.69 (m, 1H), 3.81−4.17 (m, 2H), 4.47−4.54 (m, 1H), 7.12−7.31 (m, 5H).

{**(*R*)-4-[Bis-(3-phenyl-propyl)-amino]-3-oxo-morpholin-2-yl}-acetic Acid ((*R*)-11m).** ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.59−1.90 (m, 4H), 2.56−2.87 (m, 6H), 2.84−3.06 (m, 4H), 3.23−3.30 (m, 1H), 3.47−3.60 (m, 1H), 3.70−3.82 (m, 1H), 4.00 (ddd, J = 11.9; 4.1; 2.1 Hz, 1H), 4.35−4.41 (m, 1H), 7.09−7.27 (m, 10H).

Substituted *N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[4-amino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester (12). General Procedure. To a solution of **11** (10 mM) in DMF was added PabZ × 2HCl, 1.2 equiv and DIPEA (6 equiv). HATU, 1.2 equiv, was added and the yellow solution was stirred for 100 min. The solvent was removed at room temperature using an oil pump. After purification on column chromatography, **12** was given as a white solid. Some residual DMF and tetramethylurea was evident in most cases, and thus these yields are unavailable. Solvents used for column chromatography include ethyl acetate/methanol 3:1 (**12a**, **12b**, **12c**, (*R*)-**12d**), ethyl acetate/methanol 9:1 ((*R*)-**12e**), ethyl acetate/tetrahydrofuran (3:1) ((*R*)-**12f**, (*R*)-**12k**), ethyl acetate/methanol (6:1) with 1% 15 M $NH_{3(aq)}$ ((*R*)-**12g**, (*R*)-**12h**, (*R*)-**12i**, (*S*)-**12f**, (*S*)-**12h**, (*S*)-**12k**, (*R*)-**12n**, (*S*)-**12n**, (*S*)-**12o**) and ethyl acetate/methanol (9:1) with 1% 15M $NH_{3(aq)}$ ((*R*)-**12j**, (*R*)-**12l**, (*R*)-**12m**, (*S*)-**12l**, (*S*)-**12p**, (*S*)-**12q**, (*S*)-**12r**, (*S*)-**12t**, (*S*)-**12u**).

Racemic *N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[4-(3-nitro-phenylamino)-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester (12a). See general procedure. Yield: 0.018 g, 87%, two steps from **10a**. ^{1}H NMR (methanol-d_4, 600 MHz) δ 2.84 (dd, J = 14.0; 6.3 Hz, 1H), 2.87 (dd, J = 14.0; 4.6 Hz, 1H), 3.47 (ddd, J = 11.9; 3.2; 1.8 Hz, 1H), 3.89−3.95 (m, 1H), 4.06 (ddd, J = 12.2; 10.8; 3.2 Hz, 1H), 4.14 (ddd J = 12.2; 4.5; 1.8 Hz, 1H), 4.42−4.49 (m, 2H), 4.62 (dd, J = 6.3; 4.6 Hz, 1H), 5.17 (s, 2H), 7.18 (ddd; J = 8.2; 2.3; 0.9 Hz, 1H), 7.27−7.31 (m, 1H), 7.32−7.37 (m, 2H), 7.38−7.44 (m, 5H), 7.59−7.60 (m, 1H), 7.65 (ddd, J = 8.1; 2.2; 0.9 Hz, 1H), 7.78−7.81 (m, 2H); ^{13}C NMR (methanol-d_4, 150.8 MHz) δ 38.0, 42.5, 50.6, 63.4, 66.8, 75.4, 106.8, 114.4, 118.5, 125.1, 127.3, 127.8, 127.8, 127.9, 128.0, 128.2, 128.7, 130.1, 133.2, 137.0, 143.4, 148.2, 149.4, 170.0, 170.9.

Racemic *N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[3-oxo-4-phenylamino-morpholin-2-yl]-acetamide Acid Benzyl Ester (12b). See general procedure. ^{1}H NMR (methanol-d_4, 600 MHz) δ 2.79−2.90 (m, 2H), 3.39−3.46 (m, 1H), 3.79−3.86 (m, 1H), 3.97−4.03 (m, 1H), 4.08−4.14 (m, 1H), 4.40−4.49 (m, 2H), 4.62 (dd, J = 7.5; 4.1 Hz, 1H), 5.24 (s, 2H), 6.77−6.80 (m, 2H), 6.80−6.84 (m, 1H), 6.80−6.84 (m, 2H), 6.80−6.84 (m, 3H), 7.41−7.44 (m, 4H), 7.74−7.77 (m, 2H); ^{13}C NMR (methanol-d_4, 150.8 MHz) δ 38.2, 42.5, 50.0, 63.2, 67.8, 75.3, 113.2, 120.5, 126.1, 127.5, 127.6, 128.3, 128.4, 129.2, 130.4, 136.1, 144.7, 146.3, 163.7, 168.3, 170.0, 171.3.

Racemic *N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[4-diphenylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester (12c). See general procedure. ^{1}H NMR (methanol-d_4, 600 MHz) δ 2.78−2.88 (m, 2H), 3.49−3.53 (m, 1H), 3.91−3.97 (m, 1H), 4.00−4.05 (m, 1H), 4.13−4.17 (m, 1H), 4.41 (d, J = 15.7 Hz, 1H), 4.46 (d, J = 15.7 Hz, 1H), 4.65 (dd, J = 7.5; 4.1 Hz, 1H), 5.17 (s, 2H), 6.99−7.05 (m, 2H), 7.09−7.12 (m, 2H), 7.14−7.17 (m, 2H), 7.25−7.36 (m, 7H), 7.38−7.43 (m, 4H), 7.77−7.79 (m, 2H); ^{13}C NMR (methanol-d_4, 150.8 MHz) δ 38.0, 42.5, 48.4, 63.4, 66.9, 75.8, 119.1, 119.3, 123.1, 127.4, 127.9, 127.9, 128.3, 129.2, 129.3, 133.0, 137.0, 143.3, 143.5, 143.5, 163.7, 169.3, 169.5, 171.0.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-4-benzoylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12d).** See general procedure. After column chromatography the product was further purified by preparative HPLC to give pure (*R*)-**12d** as a white solid (0.035 g, 50%, two steps from (*R*)-**10d**). $[\alpha]_D^{25}$ +77.7° (*c* 0.61 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.80 (dd, J = 15.0; 7.7 Hz, 1H), 2.92 (dd, J = 15.0; 4.1 Hz, 1H), 3.51−3.58 (m, 1H), 3.85−3.95 (m, 1H), 4.05 (ddd, J = 12.0; 10.0; 3.1 Hz, 1H), 4.13 (ddd, J = 12.0; 4.9; 2.2 Hz, 1H), 4.42 (d, J = 15.6 Hz, 1H), 4.48 (d, J = 15.6 Hz, 1H), 4.67 (dd, J = 7.7; 4.1 Hz, 1H), 5.18 (s, 2H), 7.25−7.44 (m, 7H), 7.44−7.52 (m, 2H), 7.54−7.61 (m, 1H), 7.79−7.84 (m, 2H), 7.85−7.90 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.2, 42.6, 50.9, 63.1, 66.8, 75.5, 127.3, 127.5, 127.8, 127.8, 127.9, 128.3, 128.6, 132.4, 143.4, 169.1, 171.1.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-4-*tert*-butoxycarbonylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12e).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.65−2.93 (m, 2H), 3.35−3.47 (m, 1H), 3.73−3.85 (m, 1H), 3.87−4.01 (m, 1H), 4.02−4.13 (m, 1H), 4.36−4.52 (m, 2H), 4.59 (dd, J = 8.1; 4.0 Hz, 1H), 5.19 (s, 2H), 7.25−7.47 (m, 7H), 7.75−7.86, (m, 2H).

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-4-benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12f).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.47−2.57 (m, 2H), 3.63−3.79 (m, 1H), 3.82−3.97 (m, 2H), 3.99−4.12 (m, 1H), 4.29−4.36 (m, 1H), 4.40 (s, 2H), 5.19 (s, 2H), 7.25−7.47 (m, 7H), 7.48−7.58 (m, 2H), 7.58−7.67 (m, 1H), 7.76−7.83 (m, 2H), 7.87−7.96 (m, 2H).

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-4-benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12f).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.53 (d, J = 5.9 Hz, 2H), 3.61−3.73 (m, 1H), 3.82−3.95 (m, 2H), 3.98−4.11 (m, 1H), 4.33 (t, J = 5.9 Hz, 1H), 4.41 (s, 2H), 5.26 (s, 2H), 7.31−7.47 (m, 7H), 7.50−7.58 (m, 2H), 7.59−7.67 (m, 1H), 7.73−7.79 (m, 2H), 7.88−7.94 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.0, 42.5, 52.1, 63.1, 67.9, 75.2, 127.5, 128.2, 128.25, 128.26, 128.27, 128.5, 129.0, 130.5, 133.6, 136.1, 138.3, 144.7, 160.0, 168.2, 168.3, 171.0.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-3-oxo-4-phenylacetamido-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12g).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.69−2.92 (m, 2H), 3.34−3.42 (m, 1H), 3.58 (s, 2H), 3.71−3.83 (m, 1H), 3.90−4.01 (m, 1H), 4.07 (ddd, J = 12.1; 4.5; 2.0 Hz, 1H), 4.39−4.53 (broad, 2H), 4.60 (dd, J = 7.8; 4.2 Hz, 1H), 5.29 (s, 2H), 7.19−7.41 (m, 8H), 7.41−7.52 (m, 4H), 7.72−7.81 (m, 2H);^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.2, 40.3, 42.5, 47.0, 50.7, 63.0, 68.2, 75.3, 126.9, 127.6, 128.3, 128.4, 128.5, 129.1, 129.6, 134.6, 135.8, 145.2, 168.1, 168.9, 171.2.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-3-oxo-4-phenylmethanesulfonylamino-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12h).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.85−2.91 (m, 2H), 3.49−3.60 (m, 1H), 3.82−4.07 (m, 3H), 4.38 (d, J = 13.6 Hz, 1H), 4.44 (s, 2H), 4.51−4.58 (m, 1H), 4.60 (d, J = 13.6 Hz, 1H),

5.21 (s, 2H), 7.29−7.47 (m, 12H), 7.72−7.78 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 37.9, 42.5, 52.7, 59.1, 63.5, 67.4, 75.3, 127.4, 128.0, 128.1, 128.1, 128.3, 128.4, 128.4, 129.1, 131.2, 131.8, 136.6, 144.2, 162.0, 168.8, 169.5, 170.9.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-3-oxo-4-phenylmethanesulfonylamino-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12h).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.85−2.92 (m, 2H), 3.52−3.60 (m, 1H), 3.86−4.09 (m, 3H), 4.38 (d, J = 13.6 Hz, 1H), 4.47 (s, 2H), 4.52−4.57 (m, 1H), 4.60 (d, J = 13.6 Hz, 1H), 5.29 (s, 2H), 7.29−7.51 (m, 12H), 7.71−7.78 (m, 2H).

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-3-oxo-4-(3-phenyl-ureido)-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12i).** See general procedure. Crystallization from methanol and diethyl ether yielded pure (*R*)-**12i** as a white powder (0.051 g, 91%, two steps from (*R*)-**10i**). ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.79−2.90 (m, 2H), 3.39−3.47 (m, 1H), 3.88−4.13 (m, 3H), 4.46 (s, 2H), 4.48−4.56 (m, 1H), 5.18 (s, 2H), 6.94−7.03 (m, 1H), 7.14−7.24 (m, 2H), 7.25−7.50 (m, 9H), 7.76−7.85 (m, 2H).

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-4-isopropoxycarbonylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12j).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.25 (d, J = 6.2 Hz, 6H), 2.73 (dd, J = 15.0; 8.0 Hz, 1H), 2.87 (dd, J = 15.0; 4.0 Hz, 1H), 3.39−3.47 (m, 1H), 3.75−3.86 (m, 1H), 3.88−4.00 (m, 1H), 4.08 (ddd, J = 12.0; 4.4; 2.2 Hz, 1H), 4.37−4.51 (m, 2H), 4.59 (dd, J = 8.0; 4.0 Hz, 1H), 4.90 (sept. J = 6.2 Hz, 1H), 5.23 (s, 2H), 7.26−7.39 (m, 5H), 7.39−7.46 (m, 2H), 7.76−7.82 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 21.1, 38.2, 42.6, 51.2, 63.1, 67.5, 69.9, 75.4, 127.5, 128.08, 128.10, 128.13, 128.4, 131.6, 136.5, 144.2, 156.2, 161.5, 168.8, 169.5, 171.2.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2- [(*R*)-3-oxo-4-phenethylamino-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12k).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.66−2.87 (m, 4H), 3.08−3.17 (m, 2H), 3.32−3.40 (m, 1H), 3.60−3.82 (m, 2H), 4.00 (ddd, J = 11.6; 4.0; 2.2 Hz, 1H), 4.43 (s, 2H), 4.40−4.49 (m, 1H), 5.19 (s, 2H), 7.11−7.46 (m, 12H), 7.77−7.83 (m, 2H).

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-3-oxo-4-phenethylamino-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12k).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.68−2.88 (m, 4H), 3.07−3.18 (m, 2H), 3.33−3.41 (m, 1H), 3.61−3.82 (m, 2H), 4.01 (ddd, J = 11.6; 4.0; 2.2 Hz, 1H), 4.44 (s, 2H), 4.44−4.49 (m, 1H), 5.22 (s, 2H), 7.13−7.46 (m, 12H), 7.76−7.83 (m, 2H).

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-3-oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12l).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.71−1.83 (m, 2H), 2.63−2.70 (m, 2H), 2.70−2.91 (m, 4H), 3.32−3.41 (m, 1H), 3.60−3.72 (m, 1H), 3.78−3.89 (m, 1H), 4.03 (ddd, J = 12.1; 4.3; 2.2 Hz, 1H), 4.43 (s, 2H), 4.49 (dd, J = 7.4; 4.0 Hz, 1H), 5.22 (s, 2H), 7.09−7.39 (m, 8H), 7.39−7.46 (m, 4H), 7.75−7.82 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 29.5, 33.0, 38.1, 42.5, 47.9, 48.4, 63.0, 67.4, 74.8, 125.7, 127.4, 128.06, 128.08, 128.11, 128.2, 128.3, 128.4, 131.6, 136.5, 142.0, 144.3, 161.6, 168.1, 168.8, 171.3.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-3-oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12l).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.71−1.85 (m, 2H), 2.58−2.97 (m, 6H), 3.32−3.41 (m, 1H), 3.63−3.76 (m, 1H), 3.78−3.89 (m, 1H), 3.96−4.07 (m, 1H), 4.43 (s, 2H), 4.49 (dd, J = 7.5; 4.1 Hz, 1H), 5.18 (s, 2H), 7.09−7.44 (m, 12H), 7.79−7.84 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 29.5, 33.0, 38.2, 42.5, 47.9, 48.4, 63.1, 66.9, 74.8, 125.7, 127.4, 128.0, 128.07, 128.12, 128.2, 128.3, 128.4, 133.2, 137.1, 142.0, 144.5, 168.0, 168.2, 171.3.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-{(*R*)-4-[bis-(3-phenyl-propyl)-amino]-3-oxo-morpholin-2-yl}-acetamide Acid Benzyl Ester ((*R*)-12m).** See general procedure. Yield: 98%, two steps from (*R*)-**10m**. ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.64−1.84 (m, 4H), 2.55−2.84 (m, 6H), 2.70 (dd, J = 15.0; 7.7 Hz, 1H), 2.84 (dd, J = 15.0; 3.9 Hz, 1H), 2.87−3.04 (m, 2H), 3.20−3.30 (m, 1H), 3.45−3.56 (m, 1H), 3.70−3.81 (m, 1H), 4.01 (ddd, J = 11.9; 4.1; 2.2 Hz, 1H), 4.37 (d, J = 15.6 Hz, 1H), 4.44 (d, J = 15.6 Hz, 1H), 4.46 (dd, J = 7.7; 3.9 Hz, 1H), 5.18 (s, 2H), 7.06−7.45 (m, 17H), 7.77−7.85 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 29.0, 33.1, 38.4, 42.5, 44.7, 52.9, 63.2, 66.8, 75.3, 125.7, 127.3, 127.8, 127.9, 128.2, 128.28, 128.31, 133.3, 142.2, 143.5, 169.6, 171.2.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*R*)-4-benzylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*R*)-12n).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.70 (dd, J = 15.0; 7.6 Hz, 1H), 2.83 (dd, J = 14.9; 4.0 Hz, 1H), 3.19−3.28 (m, 1H), 3.49−3.60 (m, 1H), 3.69−3.80 (m, 1H), 3.94 (ddd, J = 12.0; 4.3; 2.3 Hz, 1H), 3.99 (s, 2H), 4.45 (s, 2H), 4.48 (dd, J = 7.6; 4.0 Hz, 1H), 5.25 (s, 2H), 7.22−7.48 (m, 12H), 7.75−7.81 (m, 2H).

N-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-4-benzylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12n). See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.70 (dd, J = 15.0; 7.5 Hz, 1H), 2.81 (dd, J = 15.0; 3.9 Hz, 1H), 3.19−3.28 (m, 1H), 3.49−3.60 (m, 1H), 3.69−3.79 (m, 1H), 3.89−3.97 (m, 1H), 3.99 (s, 2H), 4.45 (s, 2H), 4.48 (dd, J = 7.5; 3.9 Hz, 1H), 5.26 (s, 2H), 7.24−7.48 (m, 12H), 7.74−7.80 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.2, 42.5, 49.1, 49.2, 52.8, 62.9, 67.9, 74.8, 127.5, 127.6, 128.21, 128.25, 128.3, 128.4, 129.2, 130.4, 136.1, 137.4, 144.8, 159.8, 168.4, 171.3.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-4-dibenzylamino-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12o).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.50 (dd, J = 14.9; 8.8 Hz, 1H), 2.59−2.68 (m, 1H), 2.84 (dd, J = 14.9; 3.5 Hz, 1H), 2.87−2.98 (m, 1H), 2.98−3.09 (m, 1H), 3.39−3.47 (m, 1H), 4.03 (d, J = 12.1 Hz, 1H), 4.11 (d, J = 12.3 Hz, 1H), 4.28 (dd, J = 8.8; 3.5 Hz, 1H), 4.33−4.49 (m, 4H), 5.17 (s, 2H), 7.23−7.44 (m, 17H), 7.75−7.81 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.4, 42.5, 51.9, 57.3, 57.7, 62.6, 66.8, 75.2, 127.3, 127.59, 127.65, 127.8, 127.9, 128.1, 128.2, 128.3, 129.5, 129.6, 133.3, 137.1, 137.6, 137.8, 143.4, 169.8, 171.4.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-4-(2,5-dimethoxy-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12p).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.54 (dd, J = 15.1; 7.4 Hz, 1H), 2.62 (dd, J = 15.1; 4.1 Hz, 1H), 3.65−3.72 (m, 1H), 3.76 (s, 3H), 3.79−3.91 (m, 2H), 3.94 (s, 1H), 3.99−4.05 (m, 1H), 4.34 (dd, J = 7.4; 4.1 Hz, 1H), 4.40 (s, 2H), 5.24 (s, 2H), 7.09 (d, J = 9.0 Hz, 1H), 7.16 (dd, J = 9.0; 3.1 Hz, 1H), 7.30−7.47 (m, 8H), 7.74−7.80 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.0, 42.5, 52.4, 55.4, 56.2, 63.2, 67.7, 75.1, 114.1, 114.9, 120.6, 127.4, 127.5, 128.1, 128.15, 128.18, 128.4, 131.1, 136.3, 144.4, 152.2, 152.9, 168.5, 168.6, 171.0.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-4-(2,4-difluoro-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12q).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.54 (dd, J = 15.2; 7.2 Hz, 1H), 2.62 (dd, J = 15.2; 4.2 Hz, 1H), 3.69−3.75 (m, 1H), 3.86−3.94 (m, 2H), 4.02−4.08 (m, 1H), 4.35 (dd, J = 7.2; 4.4 Hz, 1H), 4.41 (s, 2H), 5.25 (s, 2H), 7.03−7.18 (m, 2H), 7.28−7.46 (m, 7H), 7.75−7.81 (m, 2H), 7.87−7.96 (m, 1H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 37.8, 42.5, 52.4, 63.2, 67.7, 75.2, 105.5 (t, J = 26.2 Hz), 111.4 (dd, J = 22.3; 3.4 Hz), 127.5, 128.13, 128.16, 128.17, 131.0, 132.7 (dd, J = 10.8; 1.1 Hz), 136.3, 144.5, 168.6, 168.7, 170.9. Some signals lost in the noise (approximately 124 (dd), 161 (dd), 167 (dd)).

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-4-(4-chloro-2,5-dimethyl-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12r).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.36 (s, 3H), 2.52 (dd, J = 15.1; 7.3 Hz, 1H), 2.60 (dd, J = 15.1; 4.1 Hz, 1H), 2.66 (s, 3H), 3.64−3.70 (m, 1H), 3.80−3.91 (m, 2H), 3.97−4.07 (m, 1H), 4.30 (dd, J = 7.3; 4.1 Hz, 1H), 4.41 (s, 2H), 5.25 (s, 2H), 7.28−7.47 (m, 9H), 7.74−7.80 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 18.3, 19.1, 37.9, 42.5, 52.4, 60.4, 63.3, 67.7, 75.2, 127.5, 128.1, 128.18, 128.19, 128.4, 130.8,

132.4, 132.7, 133.7, 135.7, 136.2, 138.4, 139.2, 144.5, 168.5, 168.7, 170.9.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-4-(2,3-dihydro-benzofuran-5-sulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12t).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.53 (dd, J = 15.1; 7.4 Hz, 1H), 2.60 (dd, J = 15.1; 4.3 Hz, 1H), 3.23 (t, J = 8.8 Hz, 2H), 3.66−3.76 (m, 1H), 3.82−3.94 (m, 2H), 3.97−4.08 (m, 1H), 4.34 (dd, J = 7.4; 4.3 Hz, 1H), 4.39 (s, 2H), 4.61 (t, J = 8.8 Hz, 2H), 5.20 (s, 2H), 6.81 (d, J = 8.5 Hz, 1H), 7.28−7.45 (m, 7H), 7.63−7.68 (m, 1H), 7.70−7.73 (m, 1H), 7.75−7.81 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 28.6, 38.0, 42.5, 51.9, 63.2, 67.2, 72.5, 75.2, 109.0, 125.6, 127.4, 127.9, 127.96, 128.01, 128.3, 129.0, 129.3, 130.0, 132.5, 136.8, 143.8, 162.8, 168.1, 169.0, 170.9, 171.9.

***N*-(4-Benzyloxycarbonylamidino-benzyl)-2-[(*S*)-3-oxo-4-(2-*p*-tolyl-ethylamino)-morpholin-2-yl]-acetamide Acid Benzyl Ester ((*S*)-12u).** See general procedure. ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.27 (s, 3H), 2.66−2.86 (m, 4H), 3.05−3.13 (m, 2H), 3.32−3.39 (m, 1H), 3.60−3.70 (m, 1H), 3.71−3.81 (m, 1H), 4.00 (ddd, J = 11.7; 4.0; 2.1 Hz, 1H), 4.42 (s, 2H), 4.45 (dd, J = 7.3; 4.1 Hz, 1H), 5.19 (s, 2H), 7.04−7.12 (m, 4H), 7.25−7.44 (m, 7H), 7.77−7.82 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 19.9, 33.8, 38.1, 42.5, 48.4, 49.7, 63.0, 66.9, 74.8, 127.3, 127.87, 127.92, 128.3, 128.4, 128.7, 128.9, 133.1, 135.6, 136.4, 137.0, 143.6, 168.1, 169.3, 171.2.

Substituted *N*-(4-Carbamimidoyl-benzyl)-[4-amino-3-oxo-morpholin-2-yl]-acetamide, Acetic Acid Salt (13). General Procedure. 12 (1.4−8 mM) in alcoholic solvent was treated with palladium (10% on activated carbon (Pd(C)), 100−500 mg/mmol of **12**). The solution was stirred under hydrogen gas atmosphere for typically 1−3 h. Palladium was filtered off and the clear solution was evaporated. Purification on preparative HPLC using aqueous methanol with 1% of acetic acid as eluent followed by lyophilization gave **13** as a white powder.

Racemic *N*-(4-Amidinobenzyl)-[4-(3-amino-phenylamino)-3-oxo-morpholin-2-yl]-acetamide Mono Acetate (13a). See general procedure. **12a** (1.4 mM) and Pd(C) (450 mg/mmol of **12a**) in aqueous ethanol (95%) was hydrogenolyzed for 1 h and purified using HPLC (aqueous methanol 15%, 1% acetic acid) to yield **13a** as a white powder (0.0075 g, 48%). ^{1}H NMR (methanol-d_4, 600 MHz) δ 1.94 (s, 3H, acetate), 2.82 (dd, J = 15.1; 7.2 Hz, 1H), 2.90 (dd, J = 15.1; 4.2 Hz, 1H), 3.42−3.46 (m, 1H), 4.03 (ddd, J = 12.1; 10.8; 3.2 Hz, 1H), 4.13 (ddd, J = 12.1; 4.5; 1.8 Hz, 1H), 4.50 (s, 2H), 4.63 (dd, J = 7.2; 4.2 Hz, 1H), 6.16 (ddd, J = 7.9; 2.2; 0.8 Hz, 1H), 6.21 (t, J = 2.1 Hz, 1H), 6.25 (ddd, J = 7.9; 2.1; 0.8 Hz, 1H), 6.94 (t, J = 7.9 Hz, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H); ^{13}C NMR (methanol-d_4, 150.8 MHz) δ 38.2, 42.5, 49.8, 63.4, 75.3, 100.3, 103.6, 108.3, 127.7, 128.2, 129.7, 130.0, 145.0, 147.1, 148.6, 169.8, 171.2; HRMS m/z calcd for $C_{20}H_{25}N_6O_3^+$ (MH^+): 397.1988. Found: 397.1987. Analytical HPLC t_R = 16.9 min, 96.7% pure (isocratic 2% acetonitrile for 10 min, then gradient of 2−90% acetonitrile over 10 min @ 1 mL/min), t_R = 16.1 min, 99.4% pure (Isocratic 2% 2-propanol for 10 min, then gradient of 2−90% 2-propanol over 10 min @ 1 mL/min).

Racemic *N*-(4-Amidinobenzyl)-(3-oxo-4-phenylamino-morpholin-2-yl)-acetamide x $^1/_3$ Acetic Acid (13b). See general procedure. **12b** (7 mM) and Pd(C) (100 mg/mmol of **12b**) in aqueous ethanol (85%) was hydrogenolyzed for 90 min and purified using HPLC (aqueous methanol 30%, 1% acetic acid) to yield **13b** as a white powder (0.057 g, 59%, three steps from **10b**). ^{1}H NMR (methanol-d_4, 600 MHz) δ 1.94 (s, 1H, acetate), 2.84 (dd, J = 15.1; 7.2 Hz, 1H), 2.89 (dd, J = 15.1; 4.2 Hz, 1H), 3.44−3.48 (m, 1H), 3.85−3.91 (m, 1H), 4.02−4.08 (m, 1H), 4.12−4.17 (m, 1H), 4.49 (d, J = 15.8 Hz, 1H), 4.52 (d, J = 15.8 Hz, 1H), 4.64 (dd, J = 7.2; 4.2 Hz, 1H), 6.78−6.81 (m, 2H), 6.82−6.86 (m, 1H), 7.18−7.22 (m, 2H), 7.53−7.56 (m, 2H), 7.73−7.76 (m, 2H); ^{13}C NMR (methanol-d_4, 150.8 MHz) δ 38.1, 42.4, 49.9, 63.3, 75.3, 113.1, 120.5, 126.9, 127.8, 128.0, 129.2, 145.9, 146.3, 166.9, 170.1, 171.4; HRMS m/z calcd for $C_{20}H_{24}N_5O_3^+$ (MH^+): 382.1879. Found: 382.1874. Analytical HPLC t_R = 7.7 min, 99.4% pure (A), t_R = 6.2 min, 97.4% pure (B).

Racemic *N*-(4-Amidinobenzyl)-(4-diphenylamino-3-oxo-morpholin-2-yl)-acetamide x 0.2 Acetic Acid (13c). See general procedure. **12c** (1.4 mM) and Pd(C) (350 mg/mmol of **12c**) in aqueous ethanol (95%) was hydrogenolyzed for 45 min and purified using HPLC (aqueous methanol 50%, 1% acetic acid) to yield **13c** as a white powder (0.018 g, 71%, three steps from **10c**). ^{1}H NMR (methanol-d_4, 600 MHz) δ 1.91 (s, 0.6H, acetate), 2.84 (dd, J = 15.1; 7.0 Hz, 1H), 2.88 (dd, J = 15.1; 4.3 Hz, 1H), 3.52−3.56 (m, 1H), 3.95−4.01 (m, 1H), 4.04−4.09 (m, 1H), 4.16−4.20 (m, 1H), 4.47 (d, J = 16.0 Hz, 1H), 4.53 (d, J = 16.0 Hz, 1H), 4.67 (dd, J = 7.0; 4.3 Hz, 1H), 6.99−7.03 (m, 1H), 7.03−7.06 (m, 1H), 7.11−7.14 (m, 2H), 7.16−7.19 (m, 2H), 7.25−7.29 (m, 2H), 7.30−7.34 (m, 2H), 7.52−7.55 (m, 2H), 7.72−7.74 (m, 2H); ^{13}C NMR (methanol-d_4, 150.8 MHz) δ 37.9, 42.4, 48.4, 63.5, 75.7, 119.1, 119.3, 123.0, 123.1, 126.9, 127.88, 127.93, 129.2, 129.3, 143.3, 143.4, 145.9, 167.0, 169.6, 171.2; HRMS m/z calcd for $C_{26}H_{28}N_5O_3^+$ (MH^+): 458.2192. Found: 458.2211. Analytical HPLC t_R = 9.7 min, 98.1% pure (A), t_R = 8.6 min, 98.8% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-4-benzoylamino-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*R*)-13d).** See general procedure. (*R*)-**12d** (3 mM) and Pd(C) (500 mg/mmol of (*R*)-**12d**) in aqueous ethanol (92%) was hydrogenolyzed for 65 min and purified using HPLC (aqueous methanol 30%, 1% acetic acid) to yield (*R*)-**13d** as a white powder (0.022 g, 84%). $[\alpha]_D^{25}$ +73.9° (*c* 1.04 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.95 (s, 3H, acetate), 2.81 (dd, J = 15.0; 7.8 Hz, 1H), 2.94 (dd, J = 15.0; 4.2 Hz, 1H), 3.51−3.60 (m, 1H), 3.86−3.98 (m, 1H), 4.01−4.11 (m, 1H), 4.11−4.19 (m, 1H), 4.51 (s, 2H), 4.69 (dd, J = 7.8; 4.2 Hz, 1H), 7.46−7.63 (m, 5H), 7.73−7.78 (m, 2H), 7.85−7.90 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 21.1 (acetate), 38.3, 42.5, 50.9, 63.1, 75.5, 127.1, 127.5, 127.9, 127.9, 128.6, 132.0, 132.5, 145.8, 167.1, 167.3, 169.1, 171.3, 176.4; HRMS m/z calcd for $C_{21}H_{24}N_5O_4^+$ (MH^+): 410.1828. Found: 410.1838. Analytical HPLC t_R = 7.3 min, 100% pure (A), t_R = 6.2 min, 100% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-4-*tert*-butoxycarbonylamino-3-oxo-morpholin-2-yl]-acetamide x $^1/_2$ Acetate ((*R*)-13e).** See general procedure. (*R*)-**12e** (6 mM) and Pd(C) (200 mg/mmol of (*R*)-**12e**) in aqueous ethanol (92%) was hydrogenolyzed for 45 min and purified using HPLC (aqueous methanol 30%, 1% acetic acid) to yield (*R*)-**13e** as a white powder (0.0035 g, 39%, three steps from (*R*)-**10e**). $[\alpha]_D^{25}$ +61.1° (*c* 0.27 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.47 (s, 9H), 1.89 (s, $1^1/_2$H, acetate), 2.73 (dd, J = 15.0; 8.0 Hz, 1H), 2.89 (dd, J = 15.0; 4.0 Hz, 1H), 3.39−3.48 (m, 1H), 3.75−3.87 (m, 1H), 3.90−4.03 (m, 1H), 4.08 (ddd, J = 12.0; 4.6; 2.1 Hz, 1H), 4.49 (s, 2H), 4.60 (dd, J = 8.0; 4.0 Hz, 1H), 7.51−7.57 (m, 2H), 7.73−7.79 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 27.3, 38.2, 42.5, 51.2, 63.2, 75.4, 127.0, 127.9, 145.9, 167.1, 168.0, 171.3. One ^{13}C-signal unresolved due to low amount. HRMS m/z calcd for $C_{19}H_{28}N_5O_5^+$ (MH^+): 406.2091. Found: 410.2088.

***N*-(4-Amidinobenzyl)-[(*R*)-4-benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*R*)-13f).** See general procedure. (*R*)-**12f** (6 mM) and Pd(C) (100 mg/mmol of (*R*)-**12f**) in aqueous ethanol (92%) was hydrogenolyzed for 80 min and purified using gradient HPLC (aqueous methanol 20−100%, 1% acetic acid) to yield (*R*)-**13f** as a white powder (0.017 g, 29%, four steps from (*R*)-**9**). $[\alpha]_D^{25}$ +64.5° (*c* 1.24 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.97 (s, 3H, acetate), 2.46−2.61 (m, 2H), 3.66−3.79 (m, 1H), 3.85−3.98 (m, 2H), 3.99−4.12 (m, 1H), 4.33 (dd, J = 7.1; 4.7 Hz, 1H), 4.41 (d, J = 16.0 Hz, 1H), 4.47 (d, J = 16.0 Hz, 1H), 7.46−7.58 (m, 4H), 7.59−7.67 (m, 1H), 7.71−7.78 (m, 2H), 7.88−7.95 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 37.9, 42.4, 52.1, 63.2, 75.2, 127.0, 127.88, 127.91, 128.3, 128.9, 133.4, 138.5, 145.9, 168.1, 171.0, 174.7; HRMS m/z calcd for $C_{20}H_{24}N_5O_5S^+$ (MH^+): 446.1498. Found: 446.1504. Analytical HPLC t_R = 8.0 min, 91.9% pure (A), t_R = 6.2 min, 93.5% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-4-benzenesulfonylamino-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13f).** See general procedure. (*S*)-**12f** (8 mM) and Pd(C) (350 mg/mmol of (*S*)-**12f**) in aqueous ethanol (95%) was hydrogenolyzed

for 1 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13f** as a white powder (0.046 g, 41%, four steps from (*S*)-**9**). $[\alpha]_D^{25}$ −70.8° (methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.95 (s, 3H, acetate), 2.51 (dd, J = 15.2; 7.0 Hz, 1H), 2.57 (dd, J = 15.2, 4.7 Hz, 1H), 3.65−3.78 (m, 1H), 3.85−3.97 (m, 2H), 4.00−4.12 (m, 1H), 4.33 (dd, J = 7.0; 4.7 Hz, 1H), 4.41 (d, J = 16.0 Hz, 1H), 4.47 (d, J = 16.0 Hz, 1H), 7.46−7.58 (m, 4H), 7.60−7.67 (m, 1H), 7.71−7.77 (m, 2H), 7.88−7.94 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 37.9, 42.4, 52.1, 63.2, 75.2, 126.9, 127.89, 127.91, 128.3, 128.9, 133.5, 138.5, 145.9, 167.1, 168.1, 171.1; HRMS *m*/*z* calcd for $C_{20}H_{24}N_5O_5S^+$ (MH^+): 446.1498. Found: 446.1497. Analytical HPLC t_R = 7.9 min, 99.0% pure (A), t_R = 6.2 min, 99.9% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-3-oxo-4-phenylacetamido-morpholin-2-yl]-acetamide Mono Acetate ((*R*)-13g).** See general procedure. (*R*)-**12g** (3 mM) and Pd(C) (150 mg/mmol of (*R*)-**12g**) in aqueous ethanol (92%) was hydrogenolyzed for 60 min and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*R*)-**13g** as a white powder (0.013 g, 26%, four steps from (*R*)-**9**). $[\alpha]_D^{25}$ +50.4° (*c* 0.79 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.98 (s, 3H, acetate), 2.75 (dd, J = 15.1; 7.9 Hz, 1H), 2.89 (d, J = 15.1; 4.2 Hz, 1H), 3.36−3.43 (m, 1H), 3.59 (s, 2H), 3.73−3.84 (m, 1H), 3.98 (ddd, J = 12.1; 10.0; 3.3 Hz, 1H), 4.07 (ddd, J = 12.1; 4.7; 2.1 Hz, 1H), 4.49 (s, 2H), 4.62 /dd, J = 7.9; 4.2 Hz, 1H), 7.20−7.34 (m, 5H), 7.50−7.56 (m, 2H), 7.71−7.77 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.2, 40.3, 42.5, 50.7, 63.0, 75.3, 126.9, 127.9, 128.4, 129.1, 134.6, 145.9, 168.9, 169.4, 171.3, 174.6; HRMS *m*/*z* calcd for $C_{22}H_{26}N_5O_4^+$ (MH^+): 424.1985. Found: 424.1977. Analytical HPLC t_R = 7.6 min, 97.8% pure (A), t_R = 6.5 min, 99.4% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-3-oxo-4-phenylmethanesulfonylamino-morpholin-2-yl]-acetamide Mono Acetate ((*R*)-13h).** (*R*)-**12h** (0.051 g, 0.086 mmol) was dissolved in aqueous ethanol (92%, 25 mL). Palladium (12 mg, 10% on activated carbon) was added and the solution was stirred under hydrogen gas atmosphere for 22 h. Almost no reaction was observed, so water (1.5 mL) and more palladium (12 mg) were added. Hydrogenation was continued for another 5 h. Only partial reaction was observed so again water (3 mL) and palladium (20 mg) was added. After another 4 h palladium was filtered off and the clear solution was evaporated. Purification on preparative HPLC using aqueous methanol (gradient 30−100%) with 1% of acetic acid as eluent followed by lyophilization gave (*R*)-**13h** as a white powder (0.023 g, 43%, four steps from (*R*)-**9**). $[\alpha]_D^{25}$ +33.8° (*c* 1.05 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.98 (s, 3H, acetate), 2.87 (dd, J = 15.4; 4.5 Hz, 1H), 2.94 (dd, J = 15.4; 5.6 Hz, 1H), 3.51−3.63 (m, 1H), 3.87−4.09 (m, 3H), 4.37 (d, J = 13.7 Hz, 1H), 4.49 (s, 2H), 4.56 (dd, J = 5.6; 4.5 Hz, 1H), 4.60 (d, J = 13.7 Hz, 1H), 7.30−7.35 (m, 3H), 7.40−7.46 (m, 2H), 7.50−7.55 (m, 2H), 7.68−7.73 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 19.8 (acetate), 37.9, 42.4, 52.7, 59.0, 63.5, 75.3, 127.87, 127.88, 128.28, 128.32, 129.1, 131.1, 146.0, 169.4, 171.0; HRMS *m*/*z* calcd for $C_{21}H_{26}N_5O_5S^+$ (MH^+): 460.1655. Found: 460.1654. Analytical HPLC t_R = 8.2 min, 97.3% pure (A), t_R = 6.5 min, 98.1% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-3-oxo-4-phenylmethanesulfonylamino-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13h).** See general procedure. (*S*)-**12h** (3 mM) and Pd(C) (380 mg/mmol of (*S*)-**12h**) in aqueous ethanol (85%) was hydrogenolyzed for 1 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13h** as a white powder (0.0097 g, 8%, four steps from (*S*)-**9**). $[\alpha]_D^{25}$ −27.8° (methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.90 (s, 3H, acetate), 2.86 (dd, J = 15.4; 4.6 Hz, 1H), 2.93 (dd, J = 15.4; 5.6 Hz, 1H), 3.52−3.63 (m, 1H), 3.86−4.09 (m, 3H), 4.36 (d, J = 13.6 Hz, 1H), 4.49 (s, 2H), 4.56 (dd, J = 5.6; 4.6 Hz, 1H), 4.59 (d, J = 13.6 Hz, 1H), 7.30−7.35 (m, 3H), 7.41−7.46 (m, 2H), 7.50−7.56 (m, 2H), 7.68−7.74 (m, 2H); HRMS *m*/*z* calcd for $C_{21}H_{26}N_5O_5S^+$ (MH^+): 460.1655. Found: 460.1649. Analytical HPLC t_R = 8.2 min, 98.5% pure (A), t_R = 6.5 min, 99.7% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-3-oxo-4-(3-phenyl-ureido)-morpholin-2-yl]-acetamide Mono Acetate ((*R*)-13i).** See general procedure. (*R*)-**12i** (8 mM) and Pd(C) (150 mg/mmol of (*R*)-**12i**) in aqueous ethanol (88%)/THF 8:5 was hydrogenolyzed for 2 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*R*)-**13i** as a white powder (0.027 g, 81%). $[\alpha]_D^{25}$ +23.8° (*c* 0.20 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.94 (s, 3H, acetate), 2.87 (dd, J = 15.5; 4.4 Hz, 1H), 2.92−3.07 (broad, 1H), 3.38−3.51 (m, 1H), 3.87−4.16 (m, 3H), 4.47 (d, J = 16.0 Hz, 1H), 4.53−4.56 (broad, 1H), 4.55 (d, J = 16.0 Hz, 1H), 6.95−7.02 (m, 1H), 7.14−7.22 (m, 2H), 7.42−7.47 (m, 2H), 7.48−7.54 (m, 2H), 7.71−7.76 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 21.5 (acetate), 38.0, 42.5, 51.5, 63.3, 75.0, 120.0, 123.1, 127.1, 127.8, 127.9, 128.4, 138.8, 145.8, 156.0, 167.0, 170.3, 171.2, 177.0; HRMS *m*/*z* calcd for $C_{21}H_{25}N_6O_4^+$ (MH^+): 425.1937. Found: 425.1936. Analytical HPLC t_R = 7.6 min, 99.5% pure (A), t_R = 6.7 min, 99.7% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-4-isopropoxycarbonylamino-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*R*)-13j).** See general procedure. (*R*)-**12j** (4 mM) and Pd(C) (130 mg/mmol of (*R*)-**12j**) in aqueous ethanol (92%) was hydrogenolyzed for 1 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*R*)-**13j** as a white powder (0.040 g, 87%, four steps from (*R*)-**9**). $[\alpha]_D^{25}$ +58.9° (*c* 1.25 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.26 (d, J = 6.2 Hz, 6H), 1.98 (s, 3H, acetate), 2.74 (dd, J = 15.0; 8.1 Hz, 1H), 2.89 (dd, J = 15.0; 4.1 Hz, 1H), 3.41−3.49 (m, 1H), 3.76−3.87 (m, 1H), 3.90−4.02 (m, 1H), 4.10 (ddd, J = 12.0; 4.5; 2.2 Hz, 1H), 4.46 (d, J = 16.1 Hz, 1H), 4.52 (d, J = 16.1 Hz, 1H), 4.60 (dd, J = 8.1; 4.1 Hz, 1H), 4.91 (sept. J = 6.2 Hz, 1H), 7.51−7.56 (m, 2H), 7.74−7.79 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 19.9 (acetate), 21.1, 38.2, 42.5, 51.2, 63.1, 70.0, 75.4, 126.9, 127.93, 127.94, 145.9, 156.2, 169.5, 171.4, 174.6; HRMS *m*/*z* calcd for $C_{18}H_{26}N_5O_5^+$ (MH^+): 391.1934. Found: 391.1932. Analytical HPLC t_R = 7.1 min, 99.8% pure (A), t_R = 5.9 min, 99.5% pure (B).

***N*-(4-Amidinobenzyl)- [(*R*)-3-oxo-4-phenethylamino-morpholin-2-yl]-acetamide Monoacetate ((*R*)-13k).** See general procedure. (*R*)-**12k** (6 mM) and Pd(C) (150 mg/mmol of (*R*)-**12k**) in aqueous ethanol (92%) was hydrogenolyzed for 80 min and purified using gradient HPLC (aqueous methanol 20−100%, 1% acetic acid) to yield (*R*)-**13k** as a white powder (0.016 g, 30%, five steps from (*R*)-**9**). $[\alpha]_D^{25}$ +58.6° (*c* 1.05 methanol); ^{1}H NMR (methanol-d_4, 600 MHz) δ 1.97 (s, 3H, acetate), 2.74 (dd, J = 15.1; 7.3 Hz, 1H), 2.77−2.81 (broad, 2H), 2.84 (dd, J = 15.1; 4.0 Hz, 1H), 3.08−3.19 (broad, 2H), 3.36−3.40 (m, 1H), 3.65−3.70 (m, 1H), 3.78 (ddd, J = 12.0; 10.6; 3.2 Hz, 1H), 4.02 (ddd, J = 12.0; 4.2; 1.9 Hz, 1H), 4.46 (dd; J = 7.3; 4.0 Hz, 1H), (4.46, d, J = 15.8 Hz, 1H), 4.49 (d, J = 15.8 Hz, 1H), 7.15−7.19 (m, 1H), 7.22−7.28 (m, 4H), 7.51−7.53 (m, 2H), 7.72−7.75 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 34.3, 38.0, 42.4, 48.5, 49.6, 63.0, 74.7, 126.1, 127.0, 127.9, 128.3, 128.5, 139.6, 146.0, 167.1, 168.2, 171.4, 175.2; HRMS *m*/*z* calcd for $C_{22}H_{28}N_5O_3^+$ (MH^+): 410.2192. Found: 410.2198. Analytical HPLC t_R = 8.2 min, 98.5% pure (A), t_R = 7.3 min, 99.9% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-3-oxo-4-phenethylamino-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13k).** See general procedure. (*S*)-**12k** (6 mM) and Pd(C) (150 mg/mmol of (*S*)-**12k**) in aqueous ethanol (95%) was hydrogenolyzed for 2 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13k** as a white powder (0.0264 g, 51%, five steps from (*S*)-**9**). $[\alpha]_D^{25}$ −60.8° (*c* = 1.0 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.92 (s, 3H, acetate), 2.69−2.88 (m, 4H), 3.09−3.18 (m, 2H), 3.35−3.42 (m, 1H), 3.62−3.73 (m, 1H), 3.73−3.84 (m, 1H), 3.97−4.06 (m, 1H), 4.41−4.54 (m, 3H), 7.13−7.30 (m, 5H), 7.49−7.55 (m, 2H), 7.71−7.77 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 34.3, 38.0, 42.4, 48.4, 49.6, 63.0, 74.7, 126.1, 126.9, 127.9, 128.3, 128.5, 139.6, 146.0, 167.1, 168.2, 171.3; HRMS *m*/*z* calcd for $C_{22}H_{28}N_5O_3^+$ (MH^+): 410.2192. Found: 410.2188. Analytical HPLC t_R = 8.2 min, 98.3% pure (A), t_R = 7.3 min, 99.4% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-3-oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetamide Mono Acetate ((*R*)-13l).** See general procedure. (*R*)-**12l** (4 mM) and Pd(C) (130 mg/mmol of (*R*)-**12l**) in aqueous ethanol (92%) was hydrogenolyzed for 2 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*R*)-**13l** as a white powder (0.024 g, 49%, five steps from (*R*)-**9**). $[\alpha]_D^{25}$ +61.9° (*c* 0.98 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.72−1.84 (m, 2H), 1.97 (s, 3H, acetate), 2.62−2.94 (m, 6H), 3.35−3.44 (m, 1H), 3.62−3.73 (m, 1H), 3.85 (ddd, *J* = 12.0; 10.3; 3.3 Hz, 1H), 4.05 (ddd, *J* = 12.0; 4.3; 2.1 Hz, 1H), 4.47 (s, 2H), 4.50 (dd, *J* = 7.4; 4.1 Hz, 1H), 7.10−7.28 (m, 5H), 7.50−7.55 (m, 2H), 7.72−7.78 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 29.5, 33.0, 38.1, 42.4, 47.9, 48.4, 63.0, 74.8, 125.7, 127.0, 127.9, 128.2, 128.3, 142.0, 145.9, 168.2, 171.4; HRMS *m*/*z* calcd for $C_{23}H_{30}N_5O_3^+$ (MH^+): 424.2349. Found: 424.2338. Analytical HPLC t_R = 8.6 min, 98.5% pure (A), t_R = 8.1 min, 99.6% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-3-oxo-4-(3-phenyl-propylamino)-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13l).** See general procedure. (*S*)-**12l** (3 mM) and Pd(C) (400 mg/mmol of (*S*)-**12l**) in aqueous ethanol (92%) was hydrogenolyzed for 2 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13l** as a white powder (0.0073 g, 15%, five steps from (*S*)-**9**). $[\alpha]_D^{25}$ −64.3° (*c* = 0.42 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.73−1.85 (m, 2H), 1.96 (s, 3H, acetate), 2.64−2.93 (m, 6H), 3.35−3.43 (m, 1H), 3.63−3.74 (m, 1H), 3.86 (ddd, *J* = 12.0; 10.3; 3.3 Hz, 1H), 4.04 (ddd, *J* = 12.0; 4.2; 2.2 Hz, 1H), 4.48 (s, 2H), 4.51 (dd, *J* = 7.4; 4.1 Hz, 1H), 7.10−7.28 (m, 5H), 7.50−7.55 (m, 2H), 7.72−7.78 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 29.5, 33.0, 38.1, 42.4, 47.9, 48.5, 63.1, 74.8, 125.7, 127.0, 127.9, 128.2, 128.3, 142.0, 146.0, 168.2, 171.4; HRMS *m*/*z* calcd for $C_{23}H_{29}N_5O_3^+$ (MH^+): 424.2349. Found: 424.2349. Analytical HPLC t_R = 8.6 min, 97.8% pure (A), t_R = 8.1 min, 98.6% pure (B).

***N*-(4-Amidinobenzyl)-{(*R*)-4-[bis-(3-phenyl-propyl)-amino]-3-oxo-morpholin-2-yl}-acetamide Mono Acetate ((*R*)-13m).** See general procedure. (*R*)-**12m** (4 mM) and Pd(C) (140 mg/mmol of (*R*)-**12m**) in aqueous ethanol (92%) was hydrogenolyzed for 3 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*R*)-**13m** as a white powder (0.016 g, 75%). $[\alpha]_D^{25}$ +47.7° (*c* 0.86 methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.64−1.86 (broad, 4H), 1.94 (s, 3H, acetate), 2.54−3.04 (broad, 8H), 2.73 (dd, *J* = 15.1; 7.4 Hz, 1H), 2.85 (dd, *J* = 15.1; 4.0 Hz, 1H), 3.24−3.32 (m, 1H), 3.46−3.59 (m, 1H), 3.73−3.85 (m, 1H), 4.03 (ddd, *J* = 12.0; 3.9; 2.2 Hz, 1H), 4.39−4.52 (m, 3H), 7.07−7.29 (m, 10H), 7.47−7.54 (m, 2H), 7.70−7.77 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 21.4 (acetate), 29.1, 33.1, 38.3, 42.4, 44.5, 52.9, 63.3, 75.3, 125.7, 127.0, 127.8, 127.9, 128.2, 128.3, 142.2, 145.9, 169.6, 171.3; HRMS *m*/*z* calcd for $C_{32}H_{40}N_5O_3^+$ (MH^+): 542.3131. Found: 542.3127. Analytical HPLC t_R = 11.3 min, 99.0% pure (A), t_R = 10.4 min, 99.8% pure (B).

***N*-(4-Amidinobenzyl)-[(*R*)-4-benzylamino-3-oxo-morpholin-2-yl]-acetamide ((*R*)-13n).** Crude (*R*)-**12n** (0.037 mmol) was dissolved in DCM (3 mL) and cooled with an icebath. An ice cooled solution of anisole (0.0081 mL, 0.074 mmol) and trifluoromethanesulfonic acid (0.065 mL, 0.74 mmol) in DCM (3 mL) was added while stirring. After 15 min in ice bath the solution was neutralized with triethylamine (0.103 mL, 0.74 mmol) and evaporated. Purification was performed using preparative HPLC (gradient 30−100% aqueous methanol, 1% acetic acid). Lyophilization gave (*R*)-**13n** as a white powder (0.0146 g, quant., four steps from (*R*)-**10n**). $[\alpha]_D^{25}$ +53.7° (*c* = 0.89 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 2.73 (dd, *J* = 15.1; 7.3 Hz, 1H), 2.84 (dd, *J* = 15.1; 4.1 Hz, 1H), 3.24−3.31 (m, 1H), 3.56 (ddd, *J* = 11.9; 10.3; 4.4 Hz, 1H), 3.78 (ddd, *J* = 12.0; 10.3; 3.3 Hz, 1H), 3.96 (ddd, *J* = 12.0; 4.4; 2.2 Hz, 1H), 4.00 (s, 2H), 4.48 (s, 2H), 4.49 (dd, *J* = 7.3; 4.0 Hz, 1H), 7.25−7.42 (m, 5H), 7.50−7.56 (m, 2H), 7.72−7.78 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 38.1, 42.4, 49.1, 53.0, 62.9, 74.8, 127.1, 127.7, 127.9, 128.4, 129.2, 137.3, 146.0, 168.5, 171.4; HRMS *m*/*z* calcd for $C_{21}H_{25}N_5O_3^+$ (MH^+): 396.2036. Found: 396.2033. Analytical HPLC t_R = 7.8 min, 97.3% pure (A), t_R = 6.7 min, 98.2% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-4-benzylamino-3-oxo-morpholin-2-yl]-acetamide x $^1/_2$ Acetate ((*S*)-13n).** See procedure for (*R*)-**13n**. Crude (*S*)-**12n** (0.049 mmol) was used to give (*S*)-**13n** as a white powder (0.021 g, quant., four steps from (*S*)-**10n**). $[\alpha]_D^{25}$ −60.4° (*c* = 1.89 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.98 (s, 1$^1/_2$H), 2.72 (dd, *J* = 15.1; 7.5 Hz, 1H), 2.84 (dd, *J* = 15.1; 4.0 Hz, 1H), 3.21−3.28 (m, 1H), 3.56 (dddd, *J* = 12.1; 10.3; 4.3; 0.6 Hz, 1H), 3.76 (ddd, *J* = 12.0; 10.3; 3.3 Hz, 1H), 3.95 (ddd, *J* = 12.0; 4.3; 2.2 Hz, 1H), 4.01 (s, 2H), 4.48 (s, 2H), 4.49 (dd, *J* = 7.5; 4.0 Hz, 1H), 7.23−7.42 (m, 5H), 7.49−7.56 (m, 2H), 7.72−7.78 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 19.5, 38.1, 42.4, 49.2, 52.8, 62.9, 74.8, 126.9, 127.6, 127.9, 128.3, 129.2, 137.3, 146.0, 167.1, 168.4, 171.4; HRMS *m*/*z* calcd for $C_{21}H_{25}N_5O_3^+$ (MH^+): 396.2036. Found: 396.2032. Analytical HPLC t_R = 7.8 min, 93.1% pure (A), t_R = 6.7 min, 92.5% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-4-dibenzylamino-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13o).** See general procedure. (*S*)-**12o** (4 mM) and Pd(C) (140 mg/mmol of (*S*)-**12o**) in aqueous ethanol (92%) was hydrogenolyzed for 24 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13o** as a white powder (0.0213 g, 32%, four steps from (*S*)-**9**). $[\alpha]_D^{25}$ −52.5° (methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.96 (s, 3H, acetate), 2.52 (dd, *J* = 14.9; 8.7 Hz, 1H), 2.61−2.70 (m, 1H), 2.85 (dd, *J* = 14.9; 3.5 Hz, 1H), 2.90−3.01 (m, 1H), 3.01−3.12 (m, 1H), 3.41−3.50 (m, 1H), 4.05 (d, *J* = 11.9 Hz, 1H), 4.13 (d, *J* = 12.4 Hz, 1H), 4.28 (dd, *J* = 8.7; 3.5 Hz, 1H), 4.39−4.53 (m, 4H), 7.23−7.43 (m, 10H), 7.47−7.53 (m, 2H), 7.70−7.76 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 20.3, 38.3, 42.4, 51.9, 57.3, 57.7, 62.6, 75.2, 127.3, 127.60, 127.64, 127.8, 127.9, 128.2, 129.5, 129.6, 137.6, 137.8, 145.9, 169.8, 171.5, 175.1; HRMS *m*/*z* calcd for $C_{28}H_{31}N_5O_3^+$ (MH^+): 486.2508. Found: 486.2488. Analytical HPLC t_R = 10.8 min, 98.8% pure (A), t_R = 9.7 min, 99.2% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-4-(2,5-dimethoxy-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13p).** See general procedure. (*S*)-**12p** (4 mM) and Pd(C) (230 mg/mmol of (*S*)-**12p**) in aqueous ethanol (92%) was hydrogenolyzed for 24 min and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13p** as a white powder (0.022 g, 49%, four steps from (*S*)-**9**). $[\alpha]_D^{25}$ −50.8° (*c* = 1.04 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.98 (s, 3H, acetate), 2.55 (dd, *J* = 15.2; 7.5 Hz, 1H), 2.64 (dd, *J* = 15.2; 4.0 Hz, 1H), 3.64−3.76 (m, 1H), 3.78 (s, 3H), 3.81−3.92 (m, 2H), 3.96 (s, 3H), 3.97−4.09 (m, 1H), 4.35 (dd, *J* = 7.5; 4.0 Hz, 1H), 4.44 (s, 2H), 7.10 (d, *J* = 9.0 Hz, 1H), 7.17 (dd, *J* = 9.0; 2.9 Hz, (*R*)-1h), 7.33 (d, *J* = 2.9 Hz, 1H), 7.46−7.52 (m, 2H), 7.72−7.77 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 37.9, 42.4, 52.4, 55.4, 56.1, 63.3, 75.1, 114.1, 114.9, 120.6, 126.9, 127.5, 127.87, 127.91, 145.9, 152.2, 152.9, 168.4, 171.1; HRMS *m*/*z* calcd for $C_{22}H_{27}N_5O_7S^+$ (MH^+): 506.1709. Found: 506.1706. Analytical HPLC t_R = 8.7 min, 99.4% pure (A), t_R = 6.4 min, 99.5% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-4-(2,4-difluoro-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13q).** See general procedure. (*S*)-**12q** (4 mM) and Pd(C) (230 mg/mmol of (*S*)-**12q**) in aqueous ethanol (92%) was hydrogenolyzed for 24 min and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13q** as a white powder (0.0161 g, 37%, four steps from (*S*)-**9**). $[\alpha]_D^{25}$ −74.0° (*c* = 0.97 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.98 (s, 3H, acetate), 2.57 (dd, *J* = 15.3; 7.4 Hz, 1H), 2.64 (dd, *J* = 15.3; 4.2 Hz, 1H), 3.70−3.77 (m, 1H), 3.84−3.98 (m, 2H), 4.01−4.10 (m, 1H), 4.37 (dd, *J* = 7.4; 4.2 Hz, 1H), 4.45 (s, 2H), 7.04−7.19 (m, 2H), 7.46−7.53 (m, 2H), 7.71−7.77 (m, 2H), 7.87−7.96 (m, 1H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 20.1, 37.8, 42.4, 52.6, 63.3, 75.2, 105.5 (dd, *J* = 26.6; 25.8 Hz), 111.3 (dd, *J* = 22.3, 3.7 Hz), 124.2 (dd, *J* = 14.6; 4.0 Hz), 127.0, 127.87, 127.90, 132.7 dd, *J* = 10.9; 1.4 Hz), 145.9, 161.1 (dd, *J* = 260.2, 14.6 Hz), 166.7 (dd, *J* = 256.4; 12.5 Hz), 167.1, 168.7, 171.0, 174.7; HRMS *m*/*z* calcd for $C_{20}H_{21}F_2N_5O_5S^+$

(MH^+): 482.1310. Found: 482.1311. Analytical HPLC $t_R = 8.9$ min, 99.8% pure (A), $t_R = 6.8$ min, 99.8% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-4-(4-chloro-2,5-dimethylbenzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13r)** and ***N*-(4-Amidinobenzyl)-[(*S*)-4-(2,5-dimethyl-benzenesulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13s).** See general procedure. (*S*)-**12r** (4 mM) and Pd(C) (230 mg/mmol of (*S*)-**12r**) in aqueous ethanol (92%) was hydrogenolyzed for 29 min and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid). Two products were collected. Lyophilization gave (*S*)-**13r** as a white powder (0.0058 g, 13%, four steps from (*S*)-**9**) and (*S*)-**13s** as a white powder (0.0101 g, 24%, four steps from (*S*)-**9**). (*S*)-**13r**: $[\alpha]_D^{25}$ −64.3° (*c* = 0.4 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.94 (s, 3H, acetate), 2.38 (s, 3H), 2.53 (dd, *J* = 15.2; 7.5 Hz, 1H), 2.61 (dd, *J* = 15.2; 4.0 Hz, 1H), 2.67 (s, 3H), 3.64−3.75 (m, 1H), 3.82−3.93 (m, 2H), 3.98−4.10 (m, 1H), 4.30 (dd, *J* = 7.5; 4.0 Hz, 1H), 4.44 (s, 2H), 7.35 (s, 1H), 7.46−7.52 (m, 2H), 7.72−7.77 (m, 2H) 7.84 (s, 1H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 18.3, 19.1, 21.3, 37.9, 42.4, 52.4, 63.3, 75.2, 127.1, 127.8, 127.9, 132.4, 132.7, 133.7, 135.8, 138.4, 139.2, 145.8, 167.1, 168.6, 171.0; HRMS *m*/*z* calcd for $C_{22}H_{26}ClN_5O_5S^+$ (MH^+): 508.1421. Found: 508.1417. Analytical HPLC $t_R = 9.8$ min, 98.9% pure (A), $t_R = 8.6$ min, 97.6% pure (B). (*S*)-**13s**: $[\alpha]_D^{25}$ −61.9° (*c* = 0.78 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.95 (s, 3H, acetate), 2.36 (s, 3H), 2.50 (dd, *J* = 15.2; 7.6 Hz, 1H), 2.59 (dd, *J* = 15.2; 4.0 Hz, 1H), 2.67 (s, 3H), 3.59−3.70 (m, 1H), 3.79−3.90 (m, 2H), 3.97−4.09 (m, 1H), 4.30 (dd, *J* = 7.6; 4.0 Hz, 1H), 4.44 (s, 2H), 7.20−7.24 (m, 1H), 7.29−7.34 (m, 1H), 7.46−7.52 (m, 2H), 7.71−7.77 (m, 3H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 19.3, 19.6, 20.8, 37.9, 42.4, 52.1, 63.3, 75.2, 127.0, 127.86, 127.88, 130.5, 132.6, 134.1, 135.8, 136.1, 136.6, 145.8, 167.1, 168.5, 171.0; HRMS *m*/*z* calcd for $C_{22}H_{27}N_5O_5S^+$ (MH^+): 474.1811. Found: 474.1819. Analytical HPLC $t_R = 9.2$ min, 97.9% pure (A), $t_R = 7.4$ min, 98.0% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-4-(2,3-dihydro-benzofuran-5-sulfonylamino)-3-oxo-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13t).** See general procedure. (*S*)-**12t** (4 mM) and Pd(C) (260 mg/mmol of (*S*)-**12t**) in ethanol/THF/methanol/water 48:6:5:2 was hydrogenolyzed for 1 h and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13t** as a white powder (0.0429 g, 59%, four steps from (*S*)-**9**). $[\alpha]_D^{25}$ −73.0° (*c* = 1.2 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.98 (s, 3H, acetate), 2.55 (dd, *J* = 15.2; 7.4 Hz, 1H), 2.62 (dd, *J* = 15.2; 4.3 Hz, 1H), 3.26 (t, *J* = 8.8 Hz, 2H), 3.67−3.77 (m, 1H), 3.83−3.97 (m, 2H), 4.00−4.11 (m, 1H), 4.35 (dd, *J* = 7.4; 4.3 Hz, 1H), 4.42 (d, *J* = 16.2 Hz, 1H), 4.48 (d, *J* = 16.2 Hz, 1H), 4.63 (t, *J* = 8.8 Hz, 2H), 6.79−6.84 (m, 1H), 7.47−7.52 (m, 2H), 7.63−7.68 (m, 1H), 7.70−7.77 (m, 3H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 20.0, 28.6, 38.0, 42.4, 51.9, 63.2, 72.5, 75.1, 109.0, 125.6, 126.9, 127.88, 127.92, 129.0, 129.3, 130.0, 145.9, 167.1, 168.1, 171.1, 174.6; HRMS *m*/*z* calcd for $C_{22}H_{25}N_5O_6S^+$ (MH^+): 488.1604. Found: 488.1600. Analytical HPLC $t_R = 8.5$ min, 99.3% pure (A), $t_R = 6.4$ min, 99.7% pure (B).

***N*-(4-Amidinobenzyl)-[(*S*)-3-oxo-4-(2-*p*-tolyl-ethylamino)-morpholin-2-yl]-acetamide Mono Acetate ((*S*)-13u).** See general procedure. (*S*)-**12u** (4 mM) and Pd(C) (260 mg/mmol of (*S*)-**12u**) in aqueous ethanol (92%) was hydrogenolyzed for 25 min and purified using gradient HPLC (aqueous methanol 30−100%, 1% acetic acid) to yield (*S*)-**13u** as a white powder (0.017 g, 32%, five steps from (*S*)-**9**). $[\alpha]_D^{25}$ −74.0° (*c* = 0.97 in methanol); ^{1}H NMR (methanol-d_4, 300 MHz) δ 1.97 (s, 3H, acetate), 2.28 (s, 3H), 2.69−2.78 (m, 3H), 2.84 (dd, *J* = 15.1; 4.2 Hz, 1H), 3.05−3.15 (m, 2H), 3.34−3.41 (m, 1H), 3.62−3.72 (m, 1H), 3.74−3.84 (m, 1H), 3.98−4.06 (m, 1H), 4.41−4.53 (m, 3H), 7.05−7.13 (m, 4H), 7.49−7.55 (m, 2H), 7.71−7.77 (m, 2H); ^{13}C NMR (methanol-d_4, 75.5 MHz) δ 19.9, 20.2, 33.8, 38.1, 42.4, 48.4, 49.7, 63.0, 74.7, 127.0, 127.88, 127.90, 128.4, 128.9, 135.6, 136.4, 145.9, 167.1, 168.1, 171.3, 175.1; HRMS *m*/*z* calcd for $C_{23}H_{29}N_5O_3^+$ (MH^+): 424.2349. Found: 424.2352. Analytical HPLC $t_R = 8.7$ min, 97.8% pure (A), $t_R = 8.1$ min, 98.6% pure (B).

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(27) A different gradient was used for compound **13a** due to its high polarity.

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