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Synthesis of Potential Thrombin Inhibitors. Incorporation of Tartaric Acid Templates as P2 Proline Mimetics

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Abstract—With the objective to prepare novel non-peptidic thrombin inhibitors, bioisosteres of the inhibitory tripeptide D-Phe-Pro-Arg chain have been examined. Thus, the P1 Arg was replaced with *p*-amidinobenzylamine, an elongated homologue of the same and with 2,5-dichloro benzylamine. The P2-P3, D-Phe-Pro, was replaced with a novel tartaric acid template coupled to a series of readily available, mainly lipophilic, amines. Some of these compounds exhibit promising thrombin inhibition activity in vitro, IC_{50} ~5.9 μM.

Introduction

There are four major classes of protease enzymes; aspartic, serine, cysteine and metallo proteases, which all catalyze hydrolysis of polypeptide bonds. This catalytic activity regulates an immense number of biochemical processes in both humans and microrganisms.[1] Serine proteases (e.g., trypsin-like, α-chymotrypsin-like or elastase-like) possess an active site catalytic triad consisting of Ser 195, His 57 and Asp 102 residues.[2] The trypsin-like serine protease thrombin plays a central role in hemostasis and thrombosis. Blood coagulation (or fibrin formation) is achieved through a clotting cascade involving enzymatic activation of 13 components — factors I–XIII, where inactive precursors are sequentially activated with amplification of the original signal triggering the cascade. In the final event, thrombin converts soluble fibrinogen to an insoluble meshwork of fibrin in which blood cells are trapped, forming the clot.[3] Undesired blood clotting is the major culprit in a number of cardiovascular diseases, that is, deep venous thrombosis, pulmonary embolism, unstable angina, restenosis following angioplasty and arterial thrombosis.[4] It is thus anticipated that small molecule orally available thrombin inhibitors to regulate hemostasis and thrombosis will provide new and efficacious drugs.[2] It has been shown earlier that the tripeptide chain D-Phe-Pro-Arg, which mimics parts of thrombin's natural substrate fibrinogen, exhibits thrombin-inhibiting properties.[5] Considerable synthetic efforts have therefore been directed towards mimicking this peptide. A number of development candidates and clinical inhibitors like melagatran[6] (orally administered as the prodrug H 376/95[6]) have been developed based on this motif.[2]

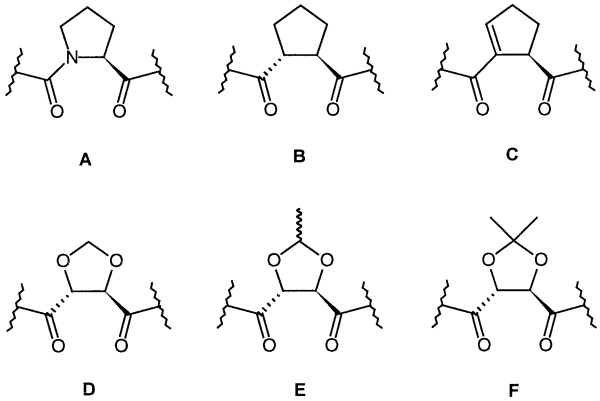

Figure 1. *N*-Acylproline (**A**) compared to some *N*-acylproline isosteres. **B** and **C** are taken from ref 8. **D**–**F** are isosteres used in this paper.

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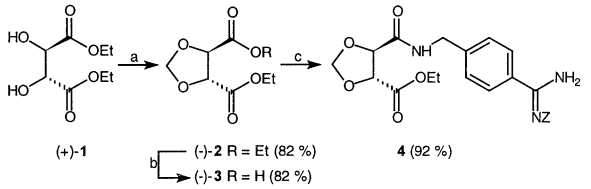


Scheme 1. Reagents: (a) diethoxymethane, BF_3, *i*-PrOAc; (b) NaOH, dioxane/water; (c) 4-(benzyloxycarbonyl)amidinobenzylamine hydrochloride, EDC, HOBt, Et_3N, DMF.

The amino acid L-proline has frequently been explored as a template and starting point for peptidomimetic inhibitor design;[7a,b,c] compare inhibitors of human immunodeficiency virus type 1 (HIV-1) protease[7d] and angiotensin-converting enzyme (ACE).[7e] There are, however, notably few reports of replacing proline with five-membered carbocyclic ring isosteres in the tripeptide type motif D-Phe-Pro-Arg. Recently, we reported on the synthesis of thrombin inhibitors containing two novel *N*-acylproline isosteres, that is, *trans*-(1*R*,2*R*)-cyclopentane-1,2-dicarboxylic acid and (2*R*)-cyclopent-2-ene-1,2-dicarboxylic acid (Fig. 1).[8]

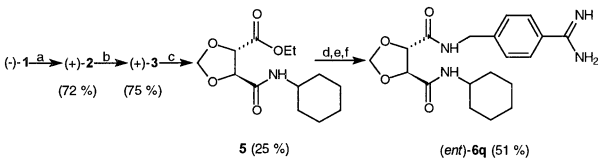
We now report on the synthesis of novel tartaric acid based *N*-acylproline isosteres, that is, *trans-(R,R)*-1,3-dioxolan-4,5-dicarboxamides[9] (Fig. 1), and the incorporation of these P2 templates in a thrombin inhibitor motif with *p*-amidinobenzylamine (Pab),[10] an elongated homologue of Pab and 2,5-dichloro benzylamine[11] in the P1 position and mainly lipophilic amines in the P3 position. Some of these compounds exhibit promising thrombin inhibition activity in vitro, $IC_{50} \sim 5.9$ μM.

Results and Discussion

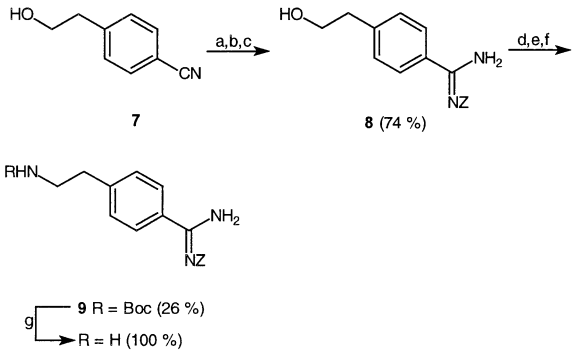
Compound (−)-**2** was prepared from readily available (+)-diethyl L-tartrate [(+)-**1**] (Scheme 1). Upon reacting compound (+)-**1** with diethoxy methane and boron trifluoride in isopropyl acetate at reflux temperature,[12] the dioxolane derivative (−)-**2**[13] was formed in 82% yield.[14]

Treatment of the diester (−)-**2** with aqueous sodium hydroxide/dioxane gave the monoacid (−)-**3** in 82% yield. Coupling of (−)-**3** with 4-(benzyloxycarbonyl)-amidinobenzylamine [Pab(Z)][10] using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt) and triethylamine in dimethylformamide afforded the monoamide **4** (92%) (Scheme 1), which was used for further couplings with P3 amines.

For the synthesis of compounds **6a**–**6t**, the monoamide (monoester) **4** was hydrolyzed by treatment with sodium hydroxide,[17] followed by coupling of the monoacid with the appropriate amine using either EDC/HOBt/Et_3N/DMF or diphenylphosphoryl azide (DPPA)/Et_3N/DMF or bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)/Et_3N/DMF, depending on the reactivity of the amines. The final deprotection step, cleavage of the benzyloxycarbonyl group by catalytic hydrogenation, generally proceeded to give the final products in excellent yields. The overall yields for the three steps were 31–88% (Table 1). The primary amide **6t** was synthesized by stirring the monoester **4** in methanol saturated with ammonia to convert the ester group to the primary amide, followed by catalytic hydrogenation to remove the protecting group, affording **6t** in 96% yield over two steps (Table 1).



Scheme 2. Reagents: (a) and (b): see Scheme 1; (c) cyclohexylamine, EDC, HOBt, Et_3N, DMF; (d) NaOH, dioxane/water; (e) 4-(benzyloxycarbonyl)amidinobenzylamine hydrochloride, EDC, HOBt, Et_3N, DMF; (f) H_2/Pd-C, EtOH.



Scheme 3. Reagents: (a) AcCl, EtOH; (b) satd NH_3 in MeOH; (c) benzyl chloroformate, K_2CO_3, THF/water; (d) MsCl, pyridine; (e) NaN_3, DMF; (f) PPh_3, Boc_2O, MeOH; (g) TFA/CH_2Cl_2.

To investigate the effect of changing stereochemistry at the two stereogenic centers of the tartrate moiety, (−)-diethyl-D-tartrate [(−)-**1**] was used as starting material. The formation of the dioxolane ring provided (+)-**2** in 72% yield. The diester (+)-**2** was hydrolyzed to give the monoacid (+)-**3** in 75% yield. Since **6q** (Tables 1 and 2) proved to be the most potent compound in the series described above we decided to use cyclohexylamine in the P3-position of this particular target molecule. The coupling of (+)-**3** with cyclohexylamine gave the monoamide **5** in 25% yield.[18] Hydrolysis, coupling with Pab(Z) and deprotection afforded the benzamidine (*ent*)-**6q** in 51% yield over three steps (Scheme 2).

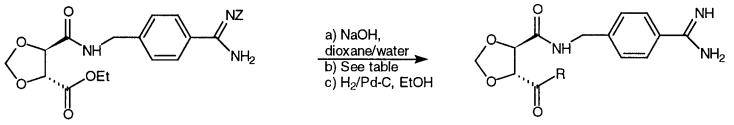
To investigate the effect of a longer benzamidine side chain in the P1 position,[19] we synthesized compound **11** (Scheme 4). The required (4-amidinophenyl)-ethylamine derivative **9** was synthesized starting from 2-(4-cyanophenyl)-ethanol (**7**) which was reacted with acetyl chloride in ethanol followed by stirring in methanol saturated with ammonia. The resulting amidine moiety was reacted with benzyl chloroformate to give the alcohol **8** in 74% yield over three steps. Compound **8** was subsequently reacted with mesyl chloride in pyridine, followed by sodium azide in dimethyl formamide. The azide group was reduced using triphenylphosphine in methanol in the presence of Boc anhydride[20] to give **9** in 26% yield over three steps (Scheme 3).

Removal of the Boc group with trifluoroacetic acid/methylene chloride, followed by coupling with the monoacid (−)-**3** gave the monoamide **10** in 50% yield. Hydrolysis, coupling with benzyl amine and deprotection afforded the benzamidine **11** in 86% yield over three steps (Scheme 4).

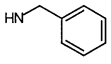
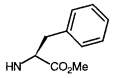
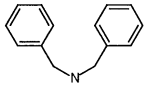
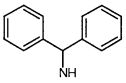
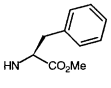
In order to investigate the effect of a different type of side chain that has been reported to fit the S1-pocket of thrombin, 2,5-dichlorobenzylamine[11] was coupled to the monoacid (−)-**3**, resulting in the monoamide **12** in 61% yield. Hydrolysis and coupling with cyclohexyl amine afforded the diamide **31** in 76% yield over two steps (Scheme 4).

Since the S2 pocket of thrombin seems to accommodate more bulk than Pro in the P2-position, (cf. inogatran[21] which contains a six-membered ring mimicking Pro in D-Phe-Pro-Arg and showing substantial thrombin inhibiting properties), slightly bulkier P2-moieties were also examined in compounds **17** and **21** (Scheme 5). Thus (+)-diethyl-L-tartrate [(+)-**1**] was reacted with either 2-methoxypropene, or 1,1-diethoxyethane and camphorsulfonic acid (CSA) in toluene[22] to form the isopropylidene diethyl tartrate **14**[23] in 74% yield and the ethylidine diethyl tartrate **18**[13b] in 80% yield, respectively. Hydrolysis afforded the monoacids **15** (47%) and **19** (69%, diastereomers 1:1), which were coupled with Pab(Z) to give the monoamides **16** and **20** in 87 and

Table 1. Structures, reaction conditions and yields for compounds **6a**–**6t**



4 **6a-6t**

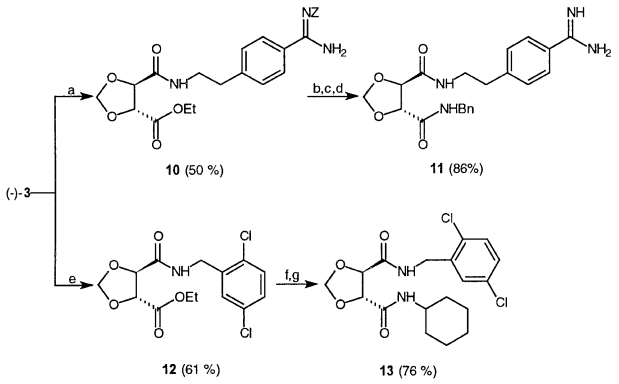
Compd	Amine R	Reagent b	Yield (%)	Compd	Amine R	Reagent b	Yield (%)
6a		A	32	**6k**		A	24
6b		A	31	**6l**		A	23
6c		B	52	**6m**		A	47
6d		A	53	**6n**		A	70
6e		A[a]	60	**6o**		C	49
6f		A[b]	50	**6p**		C	64
6g		A	42	**6q**		A	88
6h		A[a]	42	**6r**		C	71
6i		A[c]	32	**6s**		C	61
6j		A	55	**6t**	NH_2	D	96

Reagent b: A: EDC, HOBt, Et_3N, DMF; B: DPPA, Et_3N, DMF; C: BOP–Cl, Et_3N, DMF; D: Satd. NH_3 in MeOH. The hydrolysis step a was not included in the synthesis of **6t**.

[a]Benzyloxycarbonyl-protected **6d** and **6g** were hydrolyzed by treatment with NaOH in dioxane/water followed by step c to yield **6e** and **6h,** respectively.

[b]OH group protected by THP during coupling. The THP-group was removed quantitatively before step c by stirring in AcOH/THF/water 4:2:1.

[c]OH group protected by TBDMS during coupling. The TBDMS-group was removed quantitatively before step c by stirring in Bu_4NF in THF.



Scheme 4. Reagents: (a) Boc-deprotected **9**, EDC, HOBt, Et_3N, DMF; (b) NaOH, dioxane/water; (c) benzylamine, EDC, HOBt, Et_3N, DMF; (d) H_2/Pd-C, EtOH; (e) 2,5-dichlorobenzylamine, EDC, HOBt, Et_3N, DMF; (f) NaOH, dioxane/water; (g) cyclohexylamine, EDC, HOBt, Et_3N, DMF.

61% (diastereomers 1:1) yield, respectively. Subsequent hydrolysis of the monoesters, coupling with benzylamine and catalytic hydrogenation provided **17** in 35% yield over three steps and **21** as a diastereomeric mixture (1:1) in 66% yield over three steps (Scheme 5).

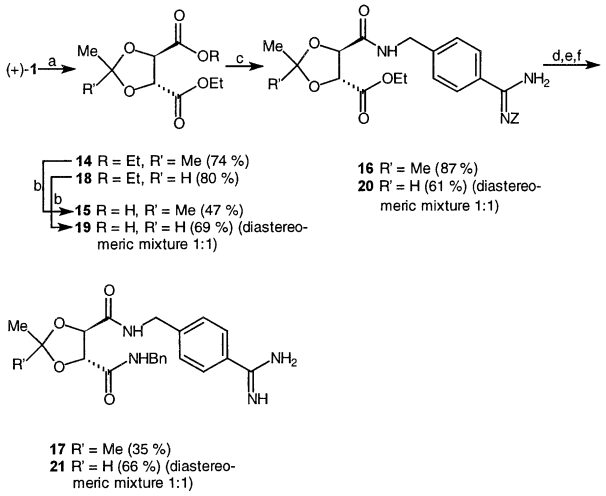
Some general remarks about the stability of the target compounds

Since all of the final compounds contain either a five-membered cyclic acetal or ketal, some instability could be expected, especially under acidic conditions. However, the vast majority of the target compounds in this report contains a methylene acetal, which requires rather harsh conditions to be cleaved. In the case of the ethylidene acetal found in **17** and the isopropylidene ketal found in **21**, reduced stability during prolonged exposure to acidic conditions must of course be expected. It must be emphasized that the primary objective of this report was to explore the in vitro thrombin inhibiting properties of these novel acetal- and ketal-containing *N*-acylproline isostere derivatives.

The stability of the benzamidine part of the target compounds is also worth some comments. We have discovered that the final compounds should not be stored as free bases, since degradation then occurs over time. Storage of the corresponding salts (e.g., acetates, hydrochlorides, etc.) is preferred.

Thrombin inhibition data

The results of the biological testing of the final products are listed in Table 2.



Scheme 5. Reagents: (a) (for compound **14**) 2-methoxypropene, CSA, toluene; (a) (for **18**) 1,1-diethoxyethane, CSA, toluene; (b) NaOH, dioxane/water; (c) 4-(benzyloxycarbonyl)amidinobenzylamine hydrochloride, EDC, HOBt, Et_3N, DMF; (d) NaOH, dioxane/water; (e) benzylamine, EDC, HOBt, Et_3N, DMF; (f) H_2/Pd-C, EtOH.

Structure–activity relationship

The compounds in this series show low to modest affinity for thrombin (Table 2), with compounds **6m**, **6n**, **6p** and **6q** having IC_{50} values around or under 10 μM. In order to provide further information on the lower affinity of these compounds compared to PPACK[24] ($IC_{50} = 0.017$ nM) compound **6m**, which was the first of the more active compounds in this series to be synthesized, was cocrystallized in complex with α-thrombin and subjected to X-ray analysis. Limited resources prevented us from performing the adequate X-ray studies on the slightly more active compounds **6p** and **6q**. A direct comparison of affinity with that of PPACK may not seem relevant since PPACK through its chloromethyl-keto group is covalently bond to His 57 and to Ser 195, as shown in Figure 2, whereas the present inhibitors do not have these interactions. However, it has been shown that other non-covalent inhibitors such as inogatran[21] ($IC_{50} = 22$ nM) (Fig. 2), which also lacks the chloromethyl-keto group, still has good affinity for thrombin and interacts with thrombin in a way very similar to PPACK.[25]

The Connolly surface map of the X-ray crystal structure of the α-thrombin–**6m** complex at 2.2 Å resolution is shown in Figure 3.

Figure 4 depicts the superimposition of the α-thrombin–**6m** and α-thrombin–PPACK X-ray crystal structures.

Table 2. Thrombin IC_{50} values (μM)

Compd	IC_{50}	Compd	IC_{50}	Compd	IC_{50}	Compd	IC_{50}	Compd	IC_{50}
6a	72	**6f**	>120	**6k**	18	**6p**	6.0	**6t**	>120
6b	>13	**6g**	42	**6l**	>120	**6q**	5.9	**11**	>120
6c	14	**6h**	>120	**6m**	8.5	(*ent*)-**6q**	48	**13**	210
6d	43	**6i**	110	**6n**	11	**6r**	>130	**17**	49
6e	42	**6j**	22	**6o**	>120	**6s**	83	**21**	46

The amidine group of **6m** forms as expected a strong salt bridge with Asp 189 in the S1-pocket, where the N–O distances are 2.96 and 2.75 Å, very similar to that of the guanidine group of PPACK. The dioxolane ring of **6m** occupies the hydrophobic S2-pocket of thrombin in a way that resembles the interaction between the P2-proline of PPACK and the S2-pocket. Additionally, the N–H of the P1–P2 amide group of **6m** makes a weak hydrogen bond (N–O distance 3.45 Å) with the carbonyl group of Ser 214, while the corresponding distance of PPACK is 2.87 Å. However, **6m** lacks the hydrogen bond network to Gly 216, which is a prominent feature of thrombin's interaction with PPACK and most non covalent bond inhibitors, including inogatran. The N–O distance of the carbonyl group of the P2–P3 amide group in **6m** to Gly 216 is 3.78 Å and the N–H of the amide group is pointing in the opposite direction compared to that of the N–H of the D-Phe of PPACK. To achieve hydrogen bonding with Gly 216 the P2-P3 amide bond in **6m** would need to adopt an energetically unfavorable *cis* geometry. Finally, the methyl group of the P3 moiety of **6m**, rather than the phenyl group, occupies the S3-pocket. Thus, the interactions with the hydrophobic P3-pocket are far from ideal and the phenyl group interacts with the surrounding water without any preferred orientation, which is verified by the poorly defined electron density of this group in the X-ray analysis. Modeling reveals that an orientation of the phenyl group into the S3-pocket requires a movement of the P2-residue of **6m**, obviously resulting in an overall less favorable interaction. Thus, the significantly lower potency of **6m** compared to that of PPACK and inogatran can be explained by the absence of a hydrogen bond network between **6m** and Gly 216, and also by the decreased hydrophobic interaction between the methyl group of **6m** and the S3-pocket compared to the interactions between the S3-pocket and the P3–D-Phe group of PPACK and the corresponding P3–D-cyclohexylalaninyl group of inogatran. Additionally, PPACK binds covalently to His 57 and Ser 195, whereas in our series of compounds these bonds, or even non-covalent interactions, are lacking. Modeling of **6l** based on the X-ray analysis of the α-thrombin–**6m** complex also explains, assuming similar conformation

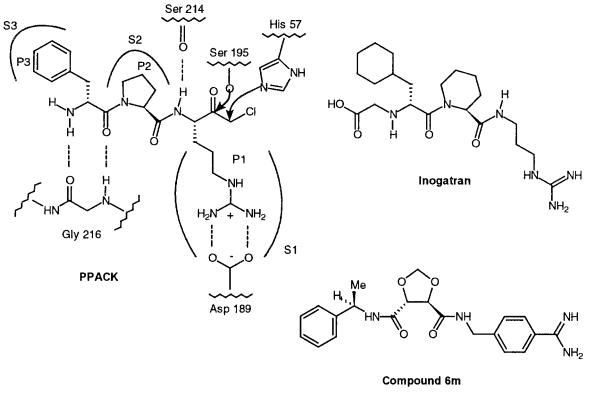


Figure 2. Structures of PPACK ($IC_{50} = 0.017$ nm), inogatran ($IC_{50} = 22$ nM) and compound **6m** ($IC_{50} = 8.5$ μM). The thrombin nomenclature and the important interactions between PPACK and thrombin are briefly outlined.

of the P2–P3 moieties, why the (*S*)-1-phenylethyl amine of **6l** will interact less favorably with thrombin compared to that of the (*R*)-1-phenylethyl amine of **6m**. In **6l** it is not possible to position the methyl group in the S3-pocket, as this would force the phenyl group in steric conflict with Trp 215. Thus, the phenyl group must be oriented towards the S3-pocket, which results in unfavorable steric interactions with the backbone of Asn 98. To avoid this the P2–P3 moiety has to move, thereby penalizing other interactions, which evidently results in the lower affinity of **6l**. This will also explain the albeit poor but reversed order of binding of the (*S*)-phenylglycine analogue **6e** ($IC_{50} = 42$ μM), which has the same relative configuration as **6l** and the (*R*)-phenylglycine analogue **6h** ($IC_{50} > 120$ μM). Assuming a conformation of **6h** with the P3-carboxyl group pointing towards the S3-pocket in a similar fashion as the methyl group of **6m** seems less plausible, and most likely the phenyl group of **6h** is oriented towards the S3-pocket even though this interaction is not optimal, resulting in a significantly decreased potency. Regarding **6e**, assuming a binding conformation with the phenyl group located in the S3-pocket as previously discussed for **6l**, the result will be an orientation of the carboxyl group towards the surrounding water. Evidently, this gives a somewhat improved potency of **6e** compared to both **6h** and **6l**.

Inhibitor **6q** ($IC_{50} = 5.9$ μM), having a cyclohexylamine as P3 substituent, is the most potent one in this series, whereas the corresponding phenyl analogue **6n** ($IC_{50} = 11.2$ μM) is slightly less active. This can probably be rationalized from a comparison of the directional vectors imposed by the sp^3 carbon of the *N*-connection of the cyclohexyl group in **6q** and the sp^2 carbon of the phenyl group in **6n**, leading to the conclusion that the cyclohexyl group seems to allow a more favorable fit in the P3-pocket. The reversed stereochemistry of the two P2-carboxy groups in (*ent*)-**6q** (Scheme 2) compared to that of **6q** resulted as predicted in a less potent although not completely inactive compound.

Most phenyl analogues prepared exhibit low affinity for thrombin. A noteworthy deviation from this is the diphenylmethyl analogue **6p** ($IC_{50} = 6.0$ μM), which in contrast to **6m** has to locate a phenyl group into the P3-pocket assuming similar binding of the P1–P2 groups. In both of the most likely conformations one of the phenyl groups will be located in the surrounding water, obviously not contributing to the affinity. However, we have in several other projects observed that the diphenylmethyl group often seems to increase potency. It appears to be a general effect of increased hydrophobicity, which for reasons not clearly understood favors the enzyme–inhibitor interaction compared to that of the free inhibitor–water interactions. The introduction of the 2,2-dimethyl and the 2-methyl groups in **17** and **21** respectively does not lower the potency compared to that of the unsubstituted dioxolane analogue **6a**. In view of the X-ray analysis of **6m** this is somewhat surprising, since the dioxolane ring very nicely fills the lipophilic S2-pocket, even better than the proline of PPACK, and an accommodation of additional groups

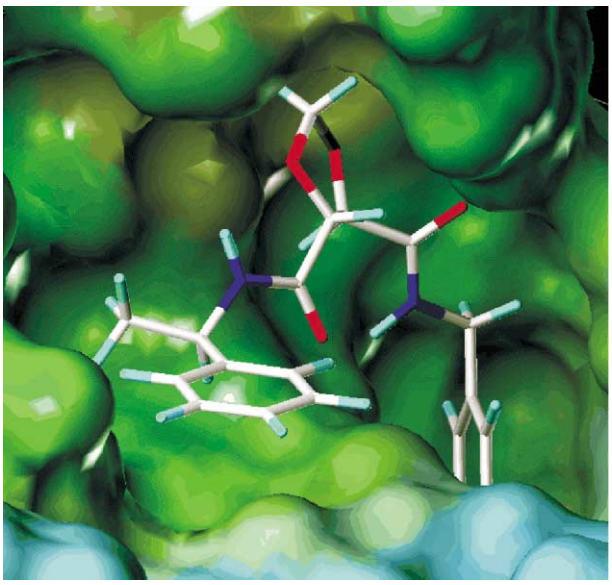


Figure 3. Connolly surface map of the X-ray structure of the α-thrombin–**6m** complex at 2.2 Å resolution.

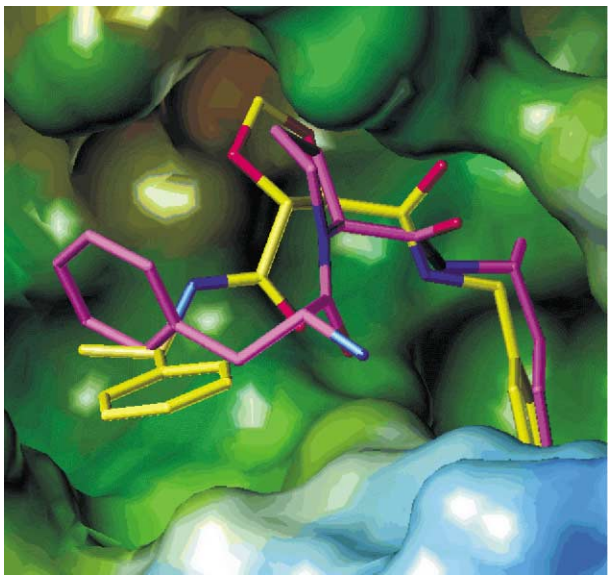


Figure 4. Connolly surface map of the X-ray structure of the α-thrombin–**6m** complex (yellow) aligned with PPACK (magenta).

Table 3. Statistics for X-ray crystallography data collection and refinement

No of measurements	105,068
No of unique reflections	27,074
Data completeness	94.5 (91.6)[a]
R_{merge}[b]	0.083 (0.377)[a]
No of atoms in refined model	2618
protein	2329
ligands	29
solvent	260
Resolution range in refinement (Å)	6.5–1.90
R.m.s. deviation for bond length (Å)	0.009
angles (°)	1.512
R_{cryst}[c]	0.221
R_{free}	0.175

[a]The numbers in parentheses are related to the data in the highest resolution shell, that is from 2.02 to 1.90 Å.
[b]$R_{merge} = S_h S_i(I(h,i) - <I(h)>)/S_h S_i I(h,i)$ where $I(h,i)$ is the intensity value of the ith measurement of h and $<I(h)>$ is the corresponding mean value of h for all i measurements of h.
[c]$R_{cryst} = S_{hkl}(|F_o - F_c|)/S_{hkl}|F_o|$. $|F_o|$ and $|F_c|$ are observed and calculated structure factor amplitudes, respectively.

does not seem possible. Obviously the substituted ring must move, but the introduction of favorable lipophilicity compensates for that.

Finally, compound **13** was prepared to see if more lipophilic P1 groups in combination with the P2 tartaric acid template would prove useful. The 2,5-dichloro benzylamine group has been incorporated in new classes of recently developed thrombin inhibitors.[11] However, this in our case results in an almost 100-fold decrease in activity of **13** compared to that of the corresponding *p*-amidinobenzylamine analogue **6q**.

Conclusion

A novel proline mimetic, that is, *trans-(R,R)*-1,3-dioxolane-4,5-dicarboxamides embedded in a thrombin inhibitor motif, has been prepared. The best of these thrombin inhibitors, having the proline templates in the P2 position, *p*-amidinobenzylamine in the P1 position and lipophilic amines in the P3 position, were **6q** ($IC_{50} = 5.9$ μM) and **6p** ($pIC_{50} = 6.0$ μM). Further optimization within this series of proline mimics should be directed towards releasing the strain in the P2-P3 amide linker by stabilization of the *cis* geometry around the P2–P3 amido group.

Experimental

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, 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. The dilutions of each test substance were analyzed consecutively, row-wise on the microtiter plate, with wash-cycles between substances to avoid cross-contamination. 2 μL of test sample was diluted with 124 μL assay buffer (0.05 mol/L Tris–HCl pH 7.4, ionic strength 0.15 adjusted with NaCl, BSA 1 g/L), 12 μL of chromogenic substrate solution (S-2366, Cromogenix, Mölndal, Sweden) and finally 12 μL of α-thrombin solution, (human α-thrombin, Sigma Chemical Co, St Louis, MO, USA; Cat No. T-6759), in buffer, was added, and the samples were mixed. The final assay concentrations were: Test substance 0.00068–13.3 μmol/L, S-2366 0.30 mmol/L and α-thrombin 0.020 NIHU/mL. The linear absorbance increase 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 which caused 50% inhibition of the thrombin activity, was calculated from a log dose versus inhibition curve.

X-ray crystallography

Human α-thrombin was purchased from Enzyme Research Laboratories, Inc., South Bend, IN, USA, and hirugen from American Diagnostica, Inc., Greenwich, CT, USA. Hirugen–thrombin complex was prepared according to the method of Skrzypczak-Jankun et al.[26] The crystallization was done as described previously.[8] The X-ray diffraction data were collected on a MAR-II imaging plate system, MAR Research, Hamburg, Germany, using Cu K_α radiation from a rotating anode. The data was reduced and scaled using DENZO and SCALEPACK[27] programs. The hirugen–α-thrombin structure previously examined in our laboratory was used in the refinement of the **6m**–thrombin complex structure. The refinement was performed using REFMAC (CCP4 package)[28] with subsequent runs of CNX.[29] Statistics for X-ray data collection and refinement are presented in Table 3.

General methods

NMR spectra were recorded on a Bruker AF 250 instrument using $CDCl_3$, methanol-d_4 or D_2O with TMS as an internal standard. The NMR measurements of the benzamidine final products were performed on the acetates unless otherwise noted. Mass spectral data was obtained in positive ion mode using a double focusing Finnigan MAT900S equipped with electrospray interface. Resolution: 5000 (10% valley definition). Two PEG references were used, one on either side (on the mass scale) of the mass of the compounds that were analyzed. Spray voltage: 1.2 kV. The infusion rate was 1.2 microliters/min. Temperature of the capillary heater: 230 °C. Optical rotations were measured in $CHCl_3$, H_2O or methanol solutions on a Perkin–Elmer 141 polarimeter. The optical rotations of the benzamidine final products were measured on their respective acetate salts. TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and charring with ethanol/sulfuric acid/acetic acid/*p*-anisaldehyde 90:3:1:2 or ethanol/acetic acid/collidine/ninhydrine 500:150:20:1 for visualisation. Column chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck). Organic phases were dried over anhydrous magnesium sulfate. Concentrations were performed under diminished pressure (1–2 kPa) at a bath temperature of 40 °C. The benzamidine final products were sent as acetates for elemental analysis, and sometimes some or all of the acetic acid was lost during drying/heating before the actual analysis. Some of the benzamidine final products gave very poor results in the elemental analyses, perhaps due to instability of the compounds. Those compounds were instead analyzed using HRMS.

General synthetic procedures

Procedure A: Monohydrolysis of diesters (typical procedure). The diester (19.7 mmol) was dissolved in water/dioxane (1:1, 100 mL), and aqueous sodium hydroxide (1 M, 20.5 mL, 20.5 mmol) was added dropwise during 30 min at room temperature. The reaction mixture was then stirred for an additional hour at room temperature. After acidification with 1 M aqueous hydrochloric acid to pH 2–3 the solution was extracted several times with dichloromethane. The combined organic layers were dried, filtered and concentrated. Finally flash column chromatography (toluene/ethyl acetate/acetic acid 20:20:1) provided the monoacid.

Procedure B: Peptide coupling (typical procedure). The monoacid (8.13 mmol) was dissolved in dimethyl formamide (50 mL). The amine (12.20 mmol) and 1-hydroxy benzotriazole (HOBt) (12.05 mmol) were added and the pH was adjusted to 7–8 with triethylamine. The mixture was cooled to 0 °C and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (12.31 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. The solvent was evaporated and the crude product was purified by flash column chromatography.

Procedure C: Ester hydrolysis + peptide coupling + deprotection of benzyloxycarbonyl protecting group (typical procedure). To a solution of the monoester (monoamide) (0.21 mmol) in dioxane/water 1:1 (5 mL) was added aqueous sodium hydroxide (1 M, 0.50 mL, 0.50 mmol) and the solution was stirred for 20 min at room temperature. After neutralization using 1 M aqueous hydrochloric acid the solvents were evaporated and coevaporated with toluene 3 times. The resulting monoacid was coupled with the appropriate amine using Procedure B. The afforded diamide was dissolved in ethanol (5 mL) and palladium on carbon (10%, 10 mg) was added and the mixture was hydrogenated (atmospheric pressure) at room temperature for 45 min. The suspension was filtered through a fine filter paper. Acetic acid (10 equivalents) was added and the solution was stirred at room temperature for 20 min. After evaporation the remainder was freeze dried in water.

Procedure D. As Procedure C except for the coupling step where diphenylphosphoryl azide (DPPA) was used instead of EDC and HOBt.

Procedure E. As Procedure C except for the coupling step where bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) was used instead of EDC and HOBt.

Synthetic experimentals

***trans*-(4*R*,5*R*)-1,3-Dioxolane-4,5-dicarboxylic acid diethyl ester [(−)-2].** To a solution of (+)-diethyl L-tartrate [(+)-**1**)] (16.61 g, 80.5 mmol) in isopropyl acetate (100 mL) were added diethoxymethane (10.1 g, 96.6 mmol) and boron trifluoride–diethyl ether complex (27.4 g, 193 mmol), and the mixture was refluxed for 7 h. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate and the layers were separated. The water phase was extracted several times with isopropyl acetate, and the combined organic layers were dried, filtered and concentrated. The crude product was purified by silica gel flash chromatography

(toluene/ethyl acetate 9:1) to give compound (−)-**2** (14.33 g, 82%) as a slightly yellow oil. (−)-**2:** α_D^{22} −75.1 (*c* 1.5, $CHCl_3$) (lit:[13c] −77.9); ^1H NMR ($CDCl_3$, 250 MHz) in agreement with ref 13c; ^{13}C NMR ($CDCl_3$, 62.9 MHz) in agreement with ref 13c.

***trans*-(4*S*,5*S*)-1,3-Dioxolane-4,5-dicarboxylic acid diethyl ester [(+)-2].** Compound (+)-**2** (3.00 g, 72%) was prepared from (−)-diethyl D-tartrate [(−)-**1**] (4.00 g, 19.4 mmol) according to the method for the preparation of (−)-**2**. (+)-**2**: α_D^{22}: +75.7 (*c* 3.4, $CHCl_3$). Anal. calcd for $C_9H_{14}O_6$: C, 49.54; H, 6.47. Found: C, 49.81; H, 6.60.

***trans*-(4*R*,5*R*)-1,3-Dioxolane-4,5-dicarboxylic acid monoethyl ester [(−)-3)].** Compound (−)-**3** (3.18 g, 82%, a slightly orange liquid) was synthesized from (−)-**2** (4.29 g, 19.7 mmol) according to Procedure A. (−)-**3**: α_D^{22} −70.7 (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.35 (t, *J* = 7.1 Hz, 3H), 4.31 (q, *J* = 7.1 Hz, 2H), 4.82 (s, 2H), 5.27 (s, 1H), 5.30 (s, 1H), 8.73 (br s, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 62.3, 76.5, 76.9, 97.6, 169.1, 174.1. Anal. calcd for $C_7H_{10}O_6$: C, 44.21; H, 5.30. Found: C, 44.01; H, 5.40.

***trans*-(4*S*,5*S*)-1,3-Dioxolane-4,5-dicarboxylic acid monoethyl ester [(+)-3].** Compound (+)-**3** (1.70 g, 75%) was prepared from (+)-**2** (2.50 g, 11.5 mmol) according to the method for the preparation of (−)-**3**. (−)-**3**: α_D^{22} +68.0 (*c* 0.9, $CHCl_3$). Anal. calcd for $C_7H_{10}O_6$: C, 44.21; H, 5.30. Found: C, 43.87; H, 5.05.

***trans*-(4*R*,5*R*)-5-{4-[Amino(benzyloxycarbonylimino)methyl]benzylaminocarbonyl} - 1,3 - dioxolane - 4 - carboxylic acid ethyl ester (4).** Compound **4** (3.0 g, 92%, a colorless solid) was prepared from (−)-**3** (1.54 g, 8.13 mmol) and 4-(benzyloxycarbonyl)amidinobenzylamine hydrochloride [Pab(Z)HCl] according to Procedure B. Chromatography mobile phase: ethyl acetate. **4:** α_D^{22} −21.3 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.31 (t, *J* = 7.1 Hz, 3H), 4.27 (q, *J* = 7.1 Hz, 2H), 4.46 (m, 2H), 4.67 (d, *J* = 3.4 Hz, 1H), 4.80 (2, *J* = 3.4 Hz, 1H), 5.14 (s, 1H), 5.20 (s, 2H), 5.23 (s, 1H), 6.97 (bs, 1H), 7.13 (bs, 1H), 7.22–7.45 (m, 7H), 7.80 (d, *J* = 8.3 Hz, 2H), 9.47 (bs, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 42.7, 62.1, 67.2, 77.2, 77.6, 96.9, 127.6, 127.8, 128.0, 128.2, 128.4, 133.8, 136.6, 142.0, 165.0, 167.8, 169.2, 169.5. Anal. calcd for $C_{23}H_{25}N_3O_7$: C, 60.65; H, 5.53; N, 9.23. Found: C, 60.85; H, 5.60; N, 9.13.

***trans*-(4*S*,5*S*)-5-Cyclohexylaminocarbonyl-1,3-dioxolane-4-carboxylic acid ethyl ester (5).** Compound **5** (185 mg, 25%, a colorless syrup) was prepared from (+)-**3** (515 mg, 2.71 mmol) and cyclohexylamine according to Procedure B. Chromatography mobile phase: ethyl acetate. **5**: α_D^{22} +56.0 (*c* 0.3, MeOH); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.10–1.50 (m, 5H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.58–1.80 (m, 3H), 1.83–2.00 (m, 2H), 3.80 (m, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.61 (d, *J* = 3.6 Hz, 1H), 4.80 (d, *J* = 3.6 Hz, 1H), 5.18 (s, 1H), 5.24 (s, 1H), 6.42 (br s, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 24.7, 25.4, 32.9, 32.0, 48.1, 61.9, 77.0, 77.7, 96.8, 167.7, 169.7. Anal. calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.40; H, 7.93; N, 5.03.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-benzyl - 1,3 - dioxolane - 4,5 - dicarboxamide (6a).** Compound **6a** (30 mg as the acetate salt, 32%) was prepared from **4** (95 mg, 0.21 mmol) and benzylamine according to Procedure C. Chromatography mobile phase after the coupling step: ethyl acetate. **6a:** α_D^{22} −8.4 (*c* 0.2, H_2O); ^1H NMR (methanol-d_4, 250 MHz) δ 1.91 (s, 3H), 4.44 (s, 2H), 4.54 (s, 2H), 4.68 (d, *J* = 4.0 Hz, 1H), 4.71 (d, *J* = 4.0 Hz, 1H), 5.21 (s, 1H), 5.23 (s, 1H), 7.21–7.38 (m, 5H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 24.0, 43.4, 43.8, 79.4, 79.5, 97.9, 128.3, 128.4, 128.5, 129.1, 129.2, 129.6, 139.6, 146.6, 168.3, 171.7, 172.1, 181.2. HRMS *m*/*z* calcd for $C_{20}H_{23}N_4O_4$ (MH^+): 383.1719. Found: 383.1727.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-benzyl-*N*^{2}-methyl-1,3-dioxolane-4,5-dicarboxamide (6b).** Compound **6b** (21 mg as the acetate salt, 31%) was prepared from **4** (68 mg, 0.15 mmol) and *N*-methyl benzylamine according to Procedure C. **6b** (rotational isomers a and b; a/b = 1.62:1): α_D^{22} −31.0 (*c* 0.8, MeOH); ^1H NMR (methanol-d_4, 250 MHz) δ 2.00 (s, 3H), 2.90 (s, 3H, isomer b), 3.11 (s, 3H, isomer a), 4.50–4.73 (m, 4H, a and b), 4.96 (d, *J* = 3.6 Hz, 1H, isomer a), 5.00 (d, *J* = 3.8 Hz, 1H, isomer b), 5.07 (d, *J* = 3.8 Hz, 1H, isomer b), 5.14 (d, *J* = 3.6 Hz, 1H, isomer a), 5.22 (s, 1H, isomer a), 5.23 (s, 1H, isomer b), 5.24 (s, 1H, isomer b), 5.27 (s, 1H, isomer a), 7.21–7.41 (m, 5H, a and b), 7.48–7.59 (m, 2H, a and b), 7.71–7.81 (m, 2H, a and b); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 20.9, 34.4, 35.2, 43.3, 52.4, 53.7, 77.9, 78.0, 78.6, 78.7, 128.0, 128.3, 128.6, 128.8, 128.9, 129.2, 129.8, 130.0, 137.6, 137.9, 146.8, 168.2, 170.3, 170.5, 172.8, 172.9, 181.3. Anal. calcd for $C_{21}H_{24}N_4O_4$1.5HOAc: C, 59.25; H, 6.22; N, 11.52. Found: C, 59.24; H, 6.27; N, 11.65.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2},*N*^{2}-dibenzyl - 1,3 - dioxolane - 4,5 - dicarboxamide (6c).** Compound **6c** (25 mg as the acetate salt, 52%) was prepared from **4** (46 mg, 0.10 mmol) and dibenzylamine according to Procedure D. **6c**: α_D^{22} −25.0 (*c* 0.2, H_2O); ^1H NMR (D_2O, 250 MHz) δ 1.90 (s, 3H), 4.43 (s, 2H), 4.47–4.70 (m, 4H), 4.84 (d, *J* = 4.3 Hz, 1H), 5.04 (d, *J* = 4.3 Hz, 1H), 5.21 (s, 1H), 5.27 (s, 1H), 7.10–7.21 (m, 5H), 7.31–7.41 (m, 7H), 7.64 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (D_2O, 62.9 MHz) δ 25.0, 44.0, 51.1, 51.9, 77.5, 79.4, 99.0, 128.5, 129.4, 129.6, 129.8, 130.6, 130.7, 137.1, 137.6, 146.0, 167.9, 172.0, 172.9, 183.3. HRMS *m*/*z* calcd for $C_{27}H_{29}N_4O_4$ (MH^+): 473.2189. Found: 473.2204.

***trans*-(4*R*,5*R*,2′*S*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-[2′-(methyl)carboxylbenzyl] - 1,3 - dioxolane - 4,5 - dicarboxamide (6d).** Compound **6d** (71 mg as the acetate salt, 53%) was prepared from **4** (125 mg, 0.27 mmol) and (*S*)-phenylglycine methyl ester according to Procedure C. **6d**: α_D^{22} −27.8 (*c* 0.2, H_2O); ^1H NMR (methanol-d_4, 250 MHz) δ 1.93 (s, 3H), 3.72 (s, 3H), 4.52 (bs, 2H), 4.70 (m, 2H), 5.25 (bs, 2H), 5.55 (s, 1H), 7.32–7.45 (m, 5H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.78 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 24.1, 43.4, 53.2, 57.9, 79.1, 79.3, 98.0, 128.7, 128.8, 129.1, 129.7, 130.0, 136.5, 136.8, 146.6, 168.2, 171.0, 171.1, 172.1, 181.7. Anal.

calcd for $C_{22}H_{24}N_4O_6 1.5H_2CO_3$: C, 52.90; H, 5.10; N, 10.50. Found: C, 53.02; H, 5.30; N, 10.61.

***trans*-(4*R*,5*R*,2′*S*)-*N*¹-{4-[Amino(imino)methyl]benzyl}-*N*²-(2′-carboxylbenzyl)-1,3-dioxolane-4,5-dicarboxamide (6e).** Compound **6e** (10 mg as the acetate salt, 100%) was prepared by dissolving benzyloxycarbonyl protected **6d** (13 mg, 0.023 mmol) in dioxane/water 1:1 followed by the addition of aqueous NaOH (1 M, 0.10 mL, 0.10 mmol). The solution was stirred at room temperature for 30 min, after which the mixture was neutralized with 1 M aqueous hydrochloric acid followed by evaporation of the solvents. The remainder was then subjected to catalytic hydrogenation as described in Procedure C. **6e**: α_D^{22} −29.4 (*c* 0.5, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.92 (s, 3H), 4.55 (s, 2H), 4.63 (m, 2H), 5.21–5.25 (m, 3H), 7.19–7.48 (m, 5H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.78 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 23.9, 43.4, 60.1, 79.1, 79.2, 97.9, 128.4, 128.5, 129.2, 129.4, 140.7, 140.8, 146.6, 168.1, 170.2, 170.4, 172.2, 182.1. HRMS *m*/*z* calcd for $C_{21}H_{23}N_4O_6$ (MH^+): 427.1618. Found: 427.1623.

***trans*-(4*R*,5*R*,2′*S*)-*N*¹-{4-[Amino(imino)methyl]benzyl}-*N*²-[2′-(hydroxymethyl)benzyl]-1,3-dioxolane-4,5-dicarboxamide (6f).** Compound **6f** (41 mg as the acetate salt, 50%) was prepared from **4** (79 mg, 0.17 mmol) and THP-protected (*S*)-phenylglycinol according to Procedure C. The THP group was removed in quantitative yield before the benzyloxycarbonyl group deprotection step, by stirring in acetic acid/tetrahydrofuran/water 4:2:1 (10 mL) for 3 h followed by evaporation of the solvents. **6f**: α_D^{22} +11.9 (*c* 1.0, MeOH); ^{1}H NMR (D_2O, 250 MHz) δ 1.93 (s, 3H), 3.90 (d, *J* = 6.1 Hz, 2H), 4.57 (s, 2H), 4.75 (bs, 2H), 5.04 (t, *J* = 6.1 Hz, 1H), 5.28 (s, 1H), 5.30 (s, 1H), 7.35–7.48 (bs, 5H), 7.52 (d, *J* = 7.8 Hz, 2H), 7.78 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (D_2O, 62.9 MHz) δ 26.0, 45.1, 58.3, 66.6, 80.3, 80.4, 99.4, 129.4, 129.6, 129.8, 130.4, 130.8, 131.6, 140.9, 146.8, 169.1, 173.5, 174.1, 184.3. HRMS *m*/*z* calcd for $C_{21}H_{25}N_4O_5$ (MH^+): 413.1825. Found: 413.1823.

***trans*-(4*R*,5*R*,2′*R*)-*N*¹-{4-[Amino(imino)methyl]benzyl}-*N*²-[2′-(methyl)carboxylbenzyl]-1,3-dioxolane-4,5-dicarboxamide (6g).** Compound **6g** (18 mg, 42%) was prepared from **4** (45 mg, 0.099 mmol) and (*R*)-phenylglycine methyl ester according to Procedure C. **6g**: α_D^{22} −23.3 (*c* 0.2, H_2O); ^{1}H NMR (the free base) (methanol-d_4, 250 MHz) δ 3.72 (s, 3H), 4.52 (s, 2H), 4.70 (m, 2H), 5.19 (s, 1H), 5.24 (s, 1H), 5.54 (s, 1H), 7.20–7.32 (m, 5H), 7.50 (d, *J* = 7.9 Hz, 2H), 7.75 (d, *J* = 7.9 Hz, 2H); ^{13}C NMR (D_2O, 62.9 MHz) δ 25.9, 45.1, 56.0, 59.6, 80.1, 80.2, 99.4, 129.5, 130.3, 130.4, 130.7, 131.9, 137.2, 137.3, 146.7, 169.1, 173.2, 174.0, 174.7, 184.2. Anal. calcd for $C_{22}H_{24}N_4O_6 0.58H_2O 1.48$-HOAc: C, 55.53; H, 5.76; N, 10.38. Found: C, 55.50; H, 5.71; N, 10.19.

***trans*-(4*R*,5*R*,2′*R*)-*N*¹-{4-[Amino(imino)methyl]benzyl}-*N*²-(2′-carboxylbenzyl)-1,3-dioxolane-4,5-dicarboxamide (6h).** Compound **6h** (210 mg as the acetate salt, 100%) was prepared from benzyloxycarbonyl protected **6g** (248 mg, 0.43 mmol) according to the method for the preparation of **6e**. **6h**: α_D^{22} −2.6 (*c* 0.2, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.92 (s, 3H), 4.53 (s, 2H), 4.60–4.66 (m, 2H), 5.20–5.27 (m, 3H), 7.20–7.45 (m, 5H), 7.51 (d, *J* = 8.3 Hz, 2H), 7.76 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 24.0, 43.4, 60.1, 79.1, 79.2, 97.9, 128.3, 128.4, 128.5, 129.2, 129.4, 140.7, 146.4, 146.5, 168.1, 170.7, 171, 172.2, 181.9. HRMS *m*/*z* calcd for $C_{21}H_{23}N_4O_6$ (MH^+): 427.1618. Found: 427.1625.

***trans*-(4*R*,5*R*,2′*R*)-*N*¹-{4-[Amino(imino)methyl]benzyl}-*N*²-[2′-(hydroxymethyl)benzyl]-1,3-dioxolane-4,5-dicarboxamide (6i).** Compound **6i** (66 mg as the acetate salt, 32%) was prepared from **4** (228 mg, 0.50 mmol) and TBDMS protected (*R*)-phenyl glycinol according to Procedure C. The TBDMS group was removed quantitatively before the benzyloxycarbonyl group deprotection by stirring in tetrahydrofuran (10 mL) and tetrabutylammonium fluoride3 H_2O (64 mg, 0.20 mmol), followed by evaporation of the solvent and filtering through a short silica plug with ethyl acetate/methanol 5:1 as eluent. **6i**: α_D^{22} −22.5 (*c* 0.4, MeOH); ^{1}H NMR (D_2O, 250 MHz) δ 1.83 (s, 3H), 3.78 (d, *J* = 6.3 Hz, 2H), 4.46 (s, 2H), 4.63 (d, *J* = 4.2 Hz, 1H), 4.71 (d, *J* = 4.2 Hz, 1H), 4.95 (t, *J* = 6.3 Hz, 1H), 5.15 (s, 1H), 5.16 (s, 1H), 7.22–7.37 (m, 5H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.67 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (D_2O, 62.9 MHz) δ 25.9, 45.1, 58.2, 66.5, 80.2, 80.3, 99.3, 129.3, 129.5, 129.6, 130.4, 130.7, 131.6, 141.0, 146.7, 169.1, 173.6, 174.0, 184.3. HRMS *m*/*z* calcd for $C_{21}H_{25}N_4O_5$ (MH^+): 413.1825. Found: 413.1822.

***trans*-(4*R*,5*R*,2′*S*)-*N*¹-{4-[Amino(imino)methyl]benzyl}-*N*²-[2′-(methyl)carboxylphenylethyl]-1,3-dioxolane-4,5-dicarboxamide (6j).** Compound **6j** (74 mg as the acetate salt, 55%) was prepared from **4** (118 mg, 0.26 mmol) and (*S*)-phenylalanine methyl ester according to Procedure C. **6j**: α_D^{22} −22.7 (*c* 0.4, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.97 (s, 3H), 3.07 (dd, *J* = 13.9, 9.1 Hz, 1H), 3.23 (dd, *J* = 13.9, 5.4 Hz, 1H), 3.71 (s, 3H), 4.52 (s, 2H), 4.55 (d, *J* = 3.9 Hz, 1H), 4.57 (d, *J* = 3.9 Hz, 1H), 4.73 (dd, *J* = 9.1, 5.4 Hz, 1H), 5.10 (s, 1H), 5.18 (s, 1H), 7.22 (m, 5H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 20.9, 37.9, 43.3, 52.9, 54.9, 79.1, 79.1, 97.9, 127.9, 128.2, 128.6, 129.2, 129.5, 130.3, 138.0, 146.7, 168.0, 171.4, 172.0, 172.9, 175.3. Anal. calcd for $C_{23}H_{26}N_4O_6 0.60$-MeOH $2.00H_2O$: C, 55.60; H, 6.41; N, 10.99. Found: C, 55.41; H, 6.56: N, 10.78.

***trans*-(4*R*,5*R*,2′*R*)-*N*¹-{4-[Amino(imino)methyl]benzyl}-*N*²-[2′-(methyl)carboxylphenylethyl]-1,3-dioxolane-4,5-dicarboxamide (6k).** Compound **6k** (32 mg as the acetate salt, 24%) was prepared from **4** (118 mg, 0.26 mmol) and (*R*)-phenylalanine methyl ester according to Procedure C. **6k**: α_D^{22} −14.9 (*c* 0.8, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.94 (s, 3H), 3.03 (dd, *J* = 13.9, 9.4 Hz, 1H), 3.27 (dd, *J* = 13.9, 5.2 Hz, 1H), 3.75 (s, 3H), 4.47 (d, *J* = 3.8 Hz, 1H), 4.62 (d, *J* = 3.8 Hz, 1H), 4.78 (dd, *J* = 9.4, 5.2 Hz, 1H), 4.91 (s, 1H), 5.14 (s, 1H), 7.17–7.33 (m, 5H), 7.51(d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 24.0, 38.0, 43.4, 52.9, 54.7, 79.1, 79.2, 97.8, 128.0, 128.2,

128.5, 129.2, 129.5, 130.3, 138.0, 146.6, 168.1, 171.7, 172.0, 172.9, 181.7. Anal. calcd for $C_{23}H_{26}N_4O_6$2.00-H_2O1.28HOAc: C, 54.10; H, 6.24; N, 9.88. Found: C, 54.10; H, 6.09; N, 9.94.

***trans*-(4*R*,5*R*,2′*S*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-(2′-methylbenzyl)-1,3-dioxolane-4,5-dicarboxamide (6l).** Compound **6l** (48 mg as the acetate salt, 23%) was prepared from **4** (213 mg, 0.47 mmol) and (*S*)-1-phenylethyl amine according to Procedure C. **6l**: α_D^{22} −45.7 (*c* 0.5, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.49 (d, *J* = 7.0 Hz, 3H), 1.97 (s, 3H), 4.48 (d, *J* = 15.7 Hz, 1H), 4.57 (d, *J* = 15.7 Hz, 1H), 4.63 (d, *J* = 4.1 Hz, 1H), 4.67 (d, *J* = 4.1 Hz, H), 5.07 (q, *J* = 7.0 Hz, 1H), 5.17 (s, 1H), 5.21 (s, 1H), 7.21–7.35 (m, 6H), 7.51 (d, *J* = 8.2 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 20.9, 22.2, 43.4, 50.2, 79.3, 79.4, 98.0, 127.0, 128.2, 129.2, 129.3, 129.4, 129.6, 144.8, 146.7, 168.1, 170.6, 172.2, 175.4. Anal. calcd for $C_{21}H_{24}N_4O_4$0.75H_2O1.66HOAc: C, 57.31; H, 6.36; N, 11.00. Found: C, 57.31; H, 6.34; N, 11.00.

***trans*-(4*R*,5*R*,2′*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-(2′-methylbenzyl)-1,3-dioxolane-4,5-dicarboxamide (6m).** Compound **6m** (80 mg as the acetate salt, 47%) was prepared from **4** (170 mg, 0.37 mmol) and (*R*)-1-phenylethylamine according to Procedure A. **6m**: α_D^{22} +5.2 (*c* 0.3, MeOH); ^{1}H NMR (D_2O, 250 MHz) δ 1.49 (d, *J* = 7.0 Hz, 3H), 1.94 (s, 3H), 4.55 (s, 2H), 4.63 (d, *J* = 4.3 Hz, 1H), 4.71 (d, *J* = 4.3 Hz, 1H), 5.0 (q, *J* = 7.0 Hz, 1H), 5.25 (s, 1H), 5.26 (s, 1H), 7.30–7.43 (m, 5H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.73 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (D_2O, 62.9 MHz) δ 23.6, 25.5, 44.9, 52.0, 80.1, 80.2, 99.2, 128.4, 129.2, 130.0, 130.3, 130.6, 131.4, 145.4, 146.7, 168.8, 172.3, 173.8, 183.3. Anal. calcd for $C_{21}H_{24}N_4O_4$8.0H_2O: C, 46.65; H, 7.46; N, 10.37. Found: C, 46.87; H, 7.50; N, 10.48.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-phenyl-1,3-dioxolane-4,5-dicarboxamide (6n).** Compound **6n** (26 mg as the acetate salt, 70%) was prepared from **4** (39 mg, 0.085 mmol) and aniline according to Procedure E. **6n**: α_D^{22} −27.8 (*c* 0.4, MeOH); ^{1}H NMR (the free base) (methanol-d_4, 250 MHz) δ 4.52 (d, *J* = 15.7 Hz, 1H), 4.61 (d, *J* = 15.7 Hz, 1H), 4.76 (d, *J* = 4.2 Hz, 1H), 4.81 (d, *J* = 4.2 Hz, 1H), 5.27 (s, 1H), 5.29 (s, 1H), 7.15–7.20 (m, 1H), 7.28–7.38(m, 2H) 7.53–7.62 (m, 4H), 7.79 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (the free base) (methanol-d_4, 62.9 MHz) δ 43.4, 79.3, 79.8, 98.1, 121.9, 126.0, 128.3, 129.0, 129.2, 129.8, 138.8, 146.7, 168.2, 169.8, 172.2. Anal. calcd for $C_{19}H_{20}N_4O_4$4.86H_2O0.36HOAc: C, 49.59; H, 6.58; N, 11.73. Found: C, 49.61; H, 6.49; N, 11.77.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-methyl-*N*^{2}-phenyl-1,3-dioxolane-4,5-dicarboxamide (6o).** Compound **6o** (25 mg as the acetate salt, 49%) was prepared from **4** (49 mg, 0.11 mmol) and *N*-methyl aniline according to Procedure E. **6o**: α_D^{22} −45.3 (*c* 0.9, MeOH); ^{1}H NMR (the free base) (methanol-d_4, 250 MHz) δ 3.31 (s, 3H), 4.40 (s, 2H), 4.47 (d, *J* = 4.7 Hz, 1H), 4.79 (d, *J* = 4.7 Hz, 1H), 5.10 (s, 1H), 5.20 (s, 1H), 7.25–7.50 (m, 7H), 7.72 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (the free base) (methanol-d_4, 62.9 MHz) δ 38.4, 43.1, 76.9, 79.2, 98.2, 128.3, 128.7, 129.1, 129.3, 129.5, 131.0, 143.7, 146.7, 170.0, 172.1, 176.3. HRMS *m*/*z* calcd for $C_{20}H_{23}N_4O_4$ (MH^+): 383.1719. Found: 383.1721.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-(2′-phenylbenzyl)-1,3-dioxolane-4,5-dicarboxamide (6p).** Compound **6p** (77 mg, 64%) was prepared from **4** (85 mg, 0.19 mmol) and 1,1-diphenyl methylamine according to Procedure E. **6p**: α_D^{22} −11.1 (*c* 0.2, MeOH); ^{1}H NMR (the free base) (methanol-d_4, 250 MHz) δ 4.50 (s, 2H), 4.68 (d, *J* = 4.3 Hz, 1H), 4.73 (d, *J* = 4.3 Hz, 1H), 5.20 (s, 1H), 5.21 (s, 1H), 6.22 (s, 1H), 7.20–7.39 (m, 10H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 2H); ^{13}C NMR (the free base) (methanol-d_4, 62.9 MHz) δ 43.4, 58.1, 79.2, 79.4, 98.0, 128.5, 128.6, 128.6, 128.8, 129.0, 129.4, 129.6, 130.4, 142.4, 142.5, 145.5, 167.9, 170.7, 172.1. Anal. calcd for $C_{26}H_{26}N_4O_4$2.10HOAc: C, 62.04; H, 5.93; N, 9.59. Found: C, 62.08; H, 5.53; N, 9.86.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-cyclohexyl-1,3-dioxolane-4,5-dicarboxamide (6q).** Compound **6q** (42 mg as the acetate salt, 88%) was prepared from **4** (51 mg, 0.11 mmol) and cyclohexylamine according to Procedure C. **6q**: α_D^{22} −16.2 (*c* 0.7, MeOH) ^{1}H NMR (the free base) (methanol-d_4, 250 MHz) δ 1.11–1.50 (m, 5H), 1.59–1.92 (m, 5H), 3.68–3.72 (m, 1H), 4.50 (d, *J* = 15.7 Hz, 1H), 4.58 (d, *J* = 15.7 Hz, 1H), 4.58 (d, *J* = 4.1 Hz, 1H), 4.65 (d, *J* = 4.1 Hz, 1H), 5.18 (s, 1H), 5.21 (s, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.79 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (the free base) (methanol-d_4, 62.9 MHz) δ 26.2, 26.5, 33.4, 33.5, 43.4, 49.9, 79.3, 79.4, 97.9, 128.3, 129.2, 129.3, 146.7, 168.1, 170.4, 172.2. HRMS *m*/*z* calcd for $C_{19}H_{27}N_4O_4$ (MH^+): 375.2032. Found: 375.2034.

***trans*-(4*S*,5*S*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-cyclohexyl-1,3-dioxolane-4,5-dicarboxamide [(ent)-6q].** Compound (*ent*)-**6q** (109 mg as the acetate salt, 51%) was prepared from **5** (134 mg, 0.49 mmol) and 4-(benzyloxycarbonyl)amidinobenzylamine hydrochloride [Pab(Z)HCl] according to Procedure C. (*ent*)-**6q**: α_D^{22}: +20.0 (*c* 0.9, MeOH). HRMS *m*/*z* calcd for $C_{19}H_{27}N_4O_4$ (MH^+): 375.2032. Found: 375.2035.

***trans*-(4*R*,5*R*)-*N*-{4-[Amino(imino)methyl]benzyl}-4-(1-piperidinylcarbonyl)-1,3-dioxolane-5-carboxamide (6r).** Compound **6r** (56 mg as the acetate salt, 71%) was prepared from **4** (83 mg, 0.18 mmol) and piperidine according to Procedure E. **6r**: α_D^{22} −33.3 (*c* 0.7, MeOH); ^{1}H NMR (the free base) (D_2O, 250 MHz) δ 1.41–1.70 (m, 6H), 3.40–3.58 (m, 4H), 4.49 (s, 2H), 4.78 (d, *J* = 3.7 Hz, 1H), 5.16 (d, *J* = 3.7 Hz, 1H), 5.19 (s, 1H), 5.22 (s, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (the free base) (methanol-d_4, 62.9 MHz) δ 25.3, 26.7, 27.4, 43.3, 44.7, 47.7, 77.8, 78.5, 98.1, 128.2, 129.2, 129.3, 146.8, 168.1, 168.2, 172.9. Anal. calcd for $C_{18}H_{24}N_4O_4$1.65H_2O1.24HOAc: C, 52.94; H, 7.00; N, 12.06. Found: C, 53.00; H, 6.72; N, 12.20.

***trans*-(4*R*,5*R*)-*N*-{4-[Amino(imino)methyl]benzyl}-4-(1-indolinylcarbonyl)-1,3-dioxolane-5-carboxamide (6s).** Compound **6s** (46 mg as the acetate salt, 61%) was

prepared from **4** (66 mg, 0.14 mmol) and indoline according to Procedure E. **6s**: α_D^{22} −45.1 (*c* 0.5, MeOH); ^{1}H NMR (the free base) (D_2O, 250 MHz) δ 2.97 (t, *J* = 8.1 Hz, 2H), 3.85–4.08 (m, 2H), 4.49 (s, 2H), 4.78 (d, *J* = 3.5 Hz, 1H), 4.98 (d, *J* = 3.5 Hz, 1H), 5.27 (s, 1H), 5.29 (s, 1H), 7.00–7.20 (m, 3H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.89 (d, *J* = 7.7 Hz, 1H); ^{13}C NMR (the free base) (D_2O, 62.9 MHz) δ 27.2, 42.1, 47.5, 76.7, 77.1, 97.0, 116.7, 124.9, 125.1, 126.3, 126.9, 127.5, 127.8, 132.6 141.2, 144.0, 165.8, 167.1, 171.1. HRMS *m*/*z* calcd for $C_{21}H_{23}N_4O_4$ (MH^+): 395.1719. Found: 395.1710.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-1,3-dioxolane-4,5-dicarboxamide (6t).** Compound **4** (83 mg, 0.18 mmol) was dissolved in methanol saturated with ammonia (10 mL) and the resulting solution was stirred at room temperature for 2 h. Volatile matters were evaporated, and the residue was applied on to a short column of silica gel and eluted with ethyl acetate/methanol (9:1). The appropriate fractions were combined and evaporated. The product was then hydrogenated according to the method described in Procedure C to give **6t** (62 mg as the acetate salt, 96%). **6t**: α_D^{22} −9.6 (*c* 0.5, MeOH); ^{1}H NMR (D_2O, 250 MHz) δ 1.84 (s, 3H), 4.44 (s, 2H), 4.61 (d, *J* = 4.2 Hz, 1H), 4.68 (d, *J* = 4.2 Hz, 1H), 5.16 (s, 1H), 5.17 (s, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.68 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (D_2O, 62.9 MHz) δ 25.0, 44.3, 79.2, 79.4, 98.4, 128.6, 129.5, 129.9, 146.0, 168.2, 173.2, 175.7, 183.0. HRMS *m*/*z* calcd for $C_{13}H_{17}N_4O_4$ (MH^+): 293.1250. Found: 293.1249.

2-[4-Amino(benzyloxycarbonylimino)methyl]ethanol (8). To an ice cooled solution of acetyl chloride (70 mL) in ethanol (140 mL) was added 2-(4-cyanophenyl)-ethanol (**7**) (4.00 g, 27.2 mmol) in portions. The solution was stirred at 0 °C for 1 h and then at room temperature for 48 h. The solvent was evaporated and residual hydrogen chloride was removed under reduced pressure. The residue was dissolved in methanol saturated with ammonia (150 mL) and stirred at room temperature overnight. The solvent was evaporated and the residue was dissolved in tetrahydrofuran/water (1:1, 100 mL). Benzyl chloroformate (4.24 mL, 29.9 mmol) and potassium carbonate (11.41 g, 81.6 mmol) were added and the resulting mixture was stirred at room temperature overnight. Diethyl ether was added, the phases were separated, the water phase was extracted with diethyl ether, and the combined organic phases were washed with water, dried, filtered, concentrated and purified by flash column chromatography (toluene/ethyl acetate 1:1) to give **8** (6.02 g, 74%) as a colorless solid. **8**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.72 (m, 2H), 3.30 (br s, 1H), 3.67 (m, 2H), 5.18 (s, 2H), 7.0–7.7 (m, 9H), 9.3 (br s, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 38.8, 62.7, 67.1, 127.5, 128.0, 128.2, 128.5, 129.2, 132.3, 136.6, 143.9, 164.5, 168.6. Anal. calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.32; H, 6.13; N, 9.37.

***N*-Butyloxycarbonyl-2-[4-amino(benzyloxycarbonylimino)methyl]ethylamine (9).** To a solution of **8** (2.39 g, 8.05 mmol) in pyridine (50 mL) was added mesyl chloride (0.73 mL, 9.66 mmol) and the mixture was stirred at 0 °C for 3 h. After addition of water (5 mL) the solvents were evaporated. The crude residue was dissolved in dichloromethane and washed with aqueous 1 M hydrogen chloride and saturated aqueous sodium hydrogen carbonate. The organic phase was dried, filtered and concentrated to give the mesylate as a colorless solid which was used without further purification. To a solution of the mesylate in dimethyl formamide (20 mL) was added sodium azide (2.62 g, 40.3 mmol) and the mixture was stirred at room temperature for 48 h. Ethyl acetate was added and the solution was washed 5 times with water. The organic phase was dried, filtered, concentrated and purified by flash column chromatography (toluene/ethyl acetate 3:1) to give 2.32 g (88%) of the azide as a colorless oil. To a solution of the azide in methanol (100 mL) was added triphenylphosphine (5.60 g, 21.3 mmol) and Boc anhydride (1.86 g, 8.54 mmol) and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated and the crude residue was purified by flash column chromatography (toluene/ethyl acetate 3:1). Recrystallization from dichloromethane/hexane gave 740 mg (26%) of **9** as colorless crystals. **9**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.42 (s, 9H), 2.84 (t, *J* = 7.0 Hz, 2H), 3.37 (t, *J* = 7.0 Hz, 2H), 4.55 (bs, 1H), 5.23 (s, 2H), 6.50 (bs, 1H), 7.20–7.50 (m, 7H), 7.81–7.92 (m, 2H), 9.50 (bs, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 28.4, 36.1, 41.5, 67.2, 79.4, 127.5, 127.9, 128.2, 128.4, 129.2, 132.7, 136.7, 143.9, 164.7, 168.0. Anal. calcd for $C_{23}H_{27}N_3O_4$: C, 66.48; H, 6.85; N, 10.57. Found: C, 66.62; H, 6.87; N, 10.44.

***trans*-(4*R*,5*R*)-5-{4-[Amino(benzyloxycarbonylimino)methyl]phenylethylaminocarbonyl}-1,3-dioxolane-4-carboxylic acid ethyl ester (10).** Compound **10** (184 mg, 50%) was prepared from (−)-**3** (149 mg, 0.78 mmol) and Boc-deprotected **9** according to Procedure B. The Boc-group in **9** was removed prior to the coupling reaction by stirring in trifluoroacetic acid/methylene choride 1:1 for 1 h at room temperature, after which the solvents were evaporated. The crude product **10** was purified by flash column chromatography (ethyl acetate/toluene 3:1). **10**: α_D^{22} −38.4 (*c* 0.4, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.30 (t, *J* = 7.1 Hz, 3H), 2.85 (t, *J* = 6.9 Hz, 2H), 3.55 (m, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.59 (d, *J* = 3.4 Hz, 1H), 4.73 (d, *J* = 3.4 Hz, 1H), 5.09 (s, 1H), 5.12 (s, 1H), 5.20 (s, 2H), 6.70 (br s, 1H), 7.20 (d, *J* = 8.2 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.26–7.48 (m, 5H), 9.51 (br s, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 35.4, 40.0, 62.1, 67.2, 77.1, 77.6, 96.8, 127.8, 128.2, 128.4, 128.6, 129.0, 132.9, 136.7, 143.1, 164.6, 168.0, 169.0, 169.5. Anal. calcd for $C_{24}H_{27}N_3O_7$: C, 61.40; H, 5.80; N, 8.95. Found: C, 61.26; H, 5.84; N, 8.82.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]phenylethyl}-*N*^{2}-benzyl-1,3-dioxolane-4,5-dicarboxamide (11).** Compound **11** (49 mg as the acetate salt, 86%) was prepared from **10** (68 mg, 0.14 mmol) and benzylamine according to Procedure C. **11**: α_D^{22} −3.8 (*c* 1.1, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.90 (s, 3H), 2.96 (t, *J* = 7.1 Hz, 2H), 3.54 (t, *J* = 7.1 Hz, 2H), 4.43 (s, 2H), 4.55 (s, 2H), 5.11 (s, 1H), 5.15 (s, 1H), 7.20–7.35 (m, 5H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (D_2O, 62.9 MHz) δ 25.9, 37.2, 42.3, 45.4, 80.1, 80.3,

99.1, 128.6, 129.8, 130.2, 130.4, 131.5, 132.5, 140.2, 148.5, 169.1, 173.4, 173.5, 184.4. HRMS *m/z* calcd for $C_{21}H_{25}N_4O_4$ (MH^+): 397.1876. Found: 397.1874.

***trans*-(4*R*,5*R*)-5-(2,5-Dichlorobenzylaminocarbonyl)-1,3-dioxolane-4-carboxylic acid ethyl ester (12).** Compound **12** (100 mg, 61%) was prepared from (−)-**3** (90 mg, 0.47 mmol) and 2,5-dichloro-benzylamine according to Procedure B. Chromatography mobile phase: Toluene/ethyl acetate 3:1. **12**: α_D^{22}−25.03 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.33 (t, *J* = 7.1 Hz, 3H), 4.29 (q, *J* = 7.1 Hz, 2H), 4.40–4.60 (m, 2H), 4.69 (d, *J* = 3.4 Hz, 1H), 4.83 (d, *J* = 3.4 Hz, 1H), 5.18 (s, 1H), 5.27 (s, 1H), 7.04 (br s, 1H), 7.18–7.33 (m, 3H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 40.9, 62.1, 76.8, 76,9, 97.0, 129.2, 129.9, 130.7, 131.8, 133.0, 136.6, 169.1, 169.5. HRMS *m/z* calcd for $C_{14}H_{16}Cl_2NO_5$ (MH^+): 348.0406. Found: 348.0419.

***trans*-(4*R*,5*R*)-*N*^{1}-(2,5-Dichlorobenzyl)-*N*^{2}-cyclohexyl-1,3-dioxolane-4,5-dicarboxamide (13).** Compound **13** (90 mg, 0.26 mmol) was dissolved in dioxane (5 mL) and sodium hydroxide (1 M, 0.50 mL) was added dropwise. The solution was stirred for 30 min at room temperature after which it was acidified with 1 M aqueous hydrogen chloride to pH 2–3 and extracted several times with dichloromethane. The organic layer was dried, filtered and concentrated. The resulting carboxylic acid was coupled with cyclohexylamine according to Procedure B to give **13** (79 mg, 76%) as a colorless solid. Chromatography mobile phase: Toluene/ethyl acetate 3:1. **13**: α_D^{22}−25.6 (*c* 3.2, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.08–1.47 (m, 5H), 1.58–1.80 (m, 3H), 1.81–1.98 (m, 2H), 3.78 (m, 1H), 4.51 (d, *J* = 5.7 Hz, 1H), 4.59 (d, *J* = 5.7 Hz, 1H), 4.54 (d, *J* = 6.2 Hz, 2H), 5.14 (s, 1H), 5.17 (s, 1H), 6.63 (d, *J* = 8.2 Hz, 1H), 7.13–7.35 (m, 3H), 7.53 (t, *J* = 6.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 24.7, 25.4, 32.8, 32.9, 40.9, 48.2, 77.4, 77.5, 96.3, 129.0, 129.7, 130.7, 131.7, 132.9, 136.8, 167.9, 169.1. HRMS *m/z* calcd for $C_{18}H_{22}Cl_2NaN_2O_4$ (MNa^+): 423.0854. Found: 423.0862.

***trans*-(4*R*,5*R*)-2,2-Dimethyl-1,3-dioxolane-4,5-dicarboxylic acid diethyl ester (14).** (+)-Diethyl L-tartrate [(+)-**1**] (2.727 g, 13.2 mmol) was dissolved in toluene (20 mL), 2-methoxy propene (2.0 mL, 21 mmol) and camphorsulfonic acid (70.0 mg, 0.30 mmol) were added and the mixture was stirred at room temperature for 5 h. The solution was washed with saturated sodium hydrogen carbonate and the organic phase was dried, filtered and concentrated. The crude product was purified by flash column chromatography (toluene/ethyl acetate 19:1) to give **14** (2.40 g, 74%) as a colorless liquid. **14**: α_D^{22}−52.5 (*c* 3.6, MeOH) (lit: [23a] −51.2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.32 (t, *J* = 7.1 Hz, 6H), 1.50 (s, 6H), 4.28 (q, *J* = 7.1 Hz, 4H), 4.78 (s, 2H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 26.4, 61.9, 77.2, 113.8, 169.7.

***trans*-(4*R*,5*R*)-2,2-Dimethyl-1,3-dioxolane-4,5-dicarboxylic acid monoethyl ester (15).** Compound **15** (691 mg, 47%) was prepared from **14** (1.678 g, 6.82 mmol) according to Procedure A. **15**: α_D^{22}−17.3 (*c* 2.1, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.35 (t, *J* = 7.1 Hz, 3H), 1.50 (s, 3H), 1.52 (s, 3H), 4.31 (q, *J* = 7.1 Hz, 2H), 4.82 (d, *J* = 5.5 Hz, 1H), 4.88 (d,*J* = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 26.3, 62.3, 76.7, 77.2, 114.2, 169.9, 174.2. Anal. calcd for $C_9H_{14}O_6$0.86HOAc: C, 47.71; H, 6.51. Found: C, 47.61; H, 6.75.

***trans*-(4*R*,5*R*)-5-{4-[Amino(benzyloxycarbonylimino)methyl]benzylaminocarb-onyl}-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid ethyl ester (16).** Compound **16** (750 mg, 87%, a colorless solid) was prepared from **15** (387 mg, 1.78 mmol) and 4-(benzyloxycarbonyl)amidinobenzylamine hydrochloride [Pab(Z)HCl] according to Procedure B. Chromatography mobile phase: Toluene/ethyl acetate 1:1. **16**: α_D^{22}−23.8 (*c* 0.6, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.32 (t, *J* = 7.1 Hz, 3H), 1.45 (s, 3H), 1.46 (s, 3H), 4.29 (q, *J* = 7.1 Hz, 2H), 4.50 (d, *J* = 6.2 Hz, 2H), 4.71 (d, *J* = 5.4 Hz, 1H), 4.81 (d, *J* = 5.4 Hz, 1H), 5.20 (s, 2H), 7.00 (t, *J* = 6.2 Hz, 1H), 7.20–7.45 (m, 7H), 7.82 (d, *J* = 8.3 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 26.2, 26.7, 42.7, 62.1, 67.2, 77.8, 113.4, 127.7, 128.0, 128.2, 128.4, 129.0, 133.9, 136.6, 142.1, 164.6, 167.7, 169.8, 170.0. Anal. calcd for $C_{25}H_{29}N_3O_7$: C, 62.10; H, 6.05; N, 8.69. Found: C, 62.14; H, 5.89; N, 8.53.

***trans*-(4*R*,5*R*)-*N*^{1}-{4-[Amino(imino)methyl]benzyl}-*N*^{2}-benzyl-2,2-dimethyl-1,3-dioxolane-4,5-dicarboxamide (17).** Compound **17** (112 mg as the acetate salt, 35%) was prepared from **16** (290 mg, 0.60 mmol) and benzylamine according to Procedure C. **17**: α_D^{22}−21.0 (*c* 0.9, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.45 (s, 3H), 1.49 (s, 3H), 1.99 (s, 3H), 4.39 (d, *J* = 14.9 Hz, 1H), 4.50 (d, *J* = 14.9 Hz, 1H), 4.53 (s, 2H), 4.67 (s, 2H), 7.22–7.35 (m, 5H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (methanol-d_4, 62.9 MHz) δ 20.8, 26.5, 26.6, 43.4, 43.8, 79.3, 79.4, 114.2, 128.3, 128.5, 129.2, 129.3, 129.5, 129.6, 139.7, 146.8, 168.1, 171.9, 172.2, 175.2. Anal. calcd for $C_{22}H_{26}N_4O_4$0.85-H_2O1.57HOAc: C, 58.06; H, 6.59; N, 10.78. Found: C, 58.10; H, 6.31; N, 10.92.

***trans*-(4*R*,5*R*)-2-Methyl-1,3-dioxolane-4,5-dicarboxylic acid diethyl ester (18).** To a solution of (+)-diethyl L-tartrate [(+)-**1**] (3.00 g, 14.5 mmol) in toluene (35 mL) were added 1,1-diethoxyethane (11.8 mL, 82.5 mmol) and camphorsulfonic acid (790 mg, 3.40 mmol). The mixture was stirred at 95 °C for 24 h, after which it was washed with saturated aqueous sodium hydrogen carbonate. The organic phase was dried, filtered and concentrated and the crude product was purified by flash column chromatography (toluene/ethyl acetate 10:1) to give **18** in 80% yield (2.68 g) as a slightly brown oil. **18**: α_D^{22}−66.3 (*c* 0.9, MeOH) (lit:[13b] −72.2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.32 (t, *J* = 7.1 Hz, 6H), 1.54 (d, *J* = 4.9 Hz, 3H), 4.31 (q, *J* = 7.1 Hz, 4H), 4.69 (d, *J* = 4.1 Hz, 1H), 4.78 (d, *J* = 4.1 Hz, 1H), 5.42 (q, *J* = 4.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.2, 19.7, 61.9, 62.0, 77.4, 77.6, 104.8, 169.2, 169.9. Anal. calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.81; H, 6.98.

***trans*-(4*R*,5*R*)-2-Methyl-1,3-dioxolane-4,5-dicarboxylic acid monoethyl ester (diastereomeric mixture) (19).** Compound **19** (183 mg, 69%, a slightly yellow oil)

(diastereomeric mixture 1:1) was prepared from **18** (300 mg, 1.29 mmol) according to Procedure A. **19**: α_D^{22} −65.7 (*c* 0.7, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.38 (t, *J* = 7.1 Hz, 3H), 1.48–1.62 (m, 3H), 4.32 (q, *J* = 7.1 Hz, 2H), 4.71–4.91 (m, 2H), 5.45 (q, *J* = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.1, 19.6, 62.3, 76.6, 77.6, 105.1, 169.1, 169.6, 174.1, 175.0. Anal. calcd for $C_8H_{12}O_6$0.32HOAc: C, 46.45; H, 5.99. Found: C, 46.45; H, 5.96.

***trans*-(4*R*,5*R*) - 5 - {4 - [Amino(benzyloxycarbonylimino)-methyl]benzylaminocarbonyl}-2-methyl-1,3-dioxolane-4-carboxylic acid ethyl ester (diastereomeric mixture 1:1) (20).** Compound **20** (375 mg, 61%, a white solid) (diastereomeric mixture 1:1) was prepared from **19** (267 mg, 1.31 mmol) and 4-(benzyloxycarbonyl)amidinobenzylamine hydrochloride [Pab(Z)HCl] according to Procedure B. Chromatography mobile phase: Ethyl acetate/toluene 20:1. **20**: α_D^{22} −18.8 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.31 (t, *J* = 7.1 Hz, 3H), 1.46 (d, *J* = 4.8 Hz, 3H), 4.18–4.31 (m, 2H), 4.37–4.50 (m, 2H), 4.61–4.81 (m, 2H), 5.13 (s, 2H), 5.35 (q, *J* = 4.8 Hz, 1H), 7.10–7.50 (m, 7H), 7.71–7.87 (m, 2H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.6, 19.6, 19.9, 42.6, 62.0, 67.1, 77.3, 77.9, 78.2, 104.2, 104.3, 127.3, 127.4, 127.9, 128.0, 128.4, 133.7, 136.6, 142.0, 164.4, 167.8, 169.4, 169.6, 169.7, 170.0. Anal. calcd for $C_{24}H_{27}N_3O_7$0.12toluene: C, 62.08; H, 5.86; N, 8.75. Found: C, 62.11; H, 5.77; N, 8.82.

***trans*-(4*R*,5*R*)-*N*^{1}-{4 - [Amino(imino)methyl]benzyl} - *N*^{2} - benzyl-2-methyl-1,3-dioxolane-4,5-dicarboxamide (diastereomeric mixture 1:1) (21).** Compound **21** (146 mg as the acetate salt, 66%) (diastereomeric mixture mixture 1:1) was prepared from **20** (228 mg, 0.49 mmol) and benzylamine according to Procedure C. **21**: α_D^{22} −16.4 (*c* 0.9, MeOH); ^{1}H NMR (methanol-d_4, 250 MHz) δ 1.46 (d, *J* = 4.8 Hz, 3H from diastereomer 1), 1.48 (d, *J* = 4.8 Hz, 3H from diastereomer 2), 1.92 (s, 3H), 4.37–4.62 (m, 4H), 4.62–4.70 (m, 2H), 5.31 (m, 1H), 7.20–7.32 (m, 5H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.81 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (the free base) (methanol-d_4, 62.9 MHz) δ 19.9, 23.6, 43.4, 43.8, 79.7, 80.0, 105.4, 128.3, 128.4, 128.5, 128.7, 129.2, 129.6, 139.7, 146.7, 168.2, 171.8, 172.0, 172.2, 172.4. HRMS *m*/*z* calcd for $C_{21}H_{25}N_4O_4$ (MH^+): 397.1876. Found: 397.1867.

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