New organofluorine building blocks: inhibition of the malarial aspartic proteases plasmepsin II and IV by alicyclic α,α-difluoroketone hydrates†

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The development of new therapeutic agents against malaria has become urgent during the past few decades, due to an increased prevalence of drug-resistant strains of malaria-causing *Plasmodium* parasites. Possible targets are the hemoglobin-degrading aspartic proteases, the plasmepsins. While acyclic α,α-difluoroketone hydrates have been introduced into peptidomimetics to bind to the catalytic Asp dyad of aspartic proteases, alicyclic derivatives were unknown. This paper describes a versatile synthesis of hydrated alicyclic α,α-difluoro-cyclopentanones and -cyclohexanones, decorated with appropriate substituents to fill the S1/S3 and the "flap-open" pocket at the enzyme active sites. Their biological activity was tested against plasmepsin II and IV, revealing an IC_{50} value (concentration of an inhibitor at which 50% maximum initial velocity is observed) of 7 μM for the best ligand. Reference inhibitors with a protonated secondary ammonium centre to address the catalytic dyad showed similar binding affinities. The X-ray crystal structure of a cyclic α,α-difluoroketone hydrate revealed the ability of these novel building blocks to participate in H-bonding networks. The hydration of difluoroketones was also investigated in solution. An exemplary study showed that the equilibrium constants for the hydration of α,α-difluorinated cyclohexanones are much higher than those for the corresponding cyclopentanones.

Introduction

The interest in finding new therapies against malaria, with 300–660 million infections annually, has increased over the past few years, since the parasite *Plasmodium falciparum* developed resistance against common malaria medicines.[1–3] Promising targets in a new strategy against malaria are the plasmepsins (PMs), aspartic proteases which participate in hemoglobin degradation within the food vacuole of the parasite during the erythrocytic stage of its life cycle.[4–6] A joint inhibition of the three plasmepsins PM I, II and IV, as well as of the structurally related histo-aspartic protease HAP (formerly known as PM III), is most likely needed to starve the parasite.[6] We recently provided evidence by *in vitro* assays that this can be accomplished with a single inhibitor.[7] High sequence homology between the four enzymes allows structure-based drug design with PM II and homology models of the other three proteins. Several X-ray crystal structures of co-crystals of PM II complexed with non-peptidic ligands reveal that inhibitors can establish H-bonds to the catalytic Asp dyad and fill two hydrophobic sub-pockets, a so-called "flap-open" pocket and the S1/S3 pocket, with lipophilic substituents.[8,9] Ligands prepared in the present study were also designed to bind to the "flap-open" conformation of the enzyme.

Our inhibitor development intended to deliver generally applicable inhibitors that can interact with aspartic proteases from mammals, parasites, fungi and retroviruses. Important representatives, which are currently pursued as drug targets, are human β-secretase (Alzheimer's disease), human renin (hypertension) and the retroviral HIV protease (AIDS).[10] Only fifteen different aspartic proteases are found in humans, making them interesting drug targets.[11] The two Asp residues in the active sites of aspartic proteases, that are crucial for catalytic activity, are part of two Asp-(Thr/Ser)-Gly sequences. Besides alcohols, amines, ammonium ions and phosphinates, acyclic hydrated α,α-difluoroketones, forming *geminal* diols, have been introduced as transition state isosteres into ligands to interact with the two Asp side chains.[11] As part of our investigations of organofluorine effects on protein–ligand binding efficacy and selectivity,[12] we became interested in extending the usage of alicyclic α,α-difluoroketone hydrates as new structural motifs to bind to the catalytic Asp dyad of aspartic proteases. The *geminal* diol mimics the transition state for amide cleavage well, and establishes a similar hydrogen bond pattern. So far, all known hydrated α,α-difluoroketones are acyclic and have been introduced into peptidomimetic inhibitors.[13–19] This paper reports the synthesis and biological activity of the first hydrated α,α-difluoro-cyclopentanone and -cyclohexanone templates bearing suitable exit-vectors to address the "flap-open" and S1/S3 pockets of PMs.[7]

In order to analyze the binding patterns of hydrated α,α-difluoroketones, the RCSB protein data bank (PDB) was searched with the program Relibase.[20] In a total of eight X-ray crystal

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† Electronic supplementary information (ESI) available: X-Ray crystal structure of (±)-**11** and preparation of compounds (±)-**2**–**4**, **5** and **6**. CCDC reference numbers 729039 and 729040. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b908489d

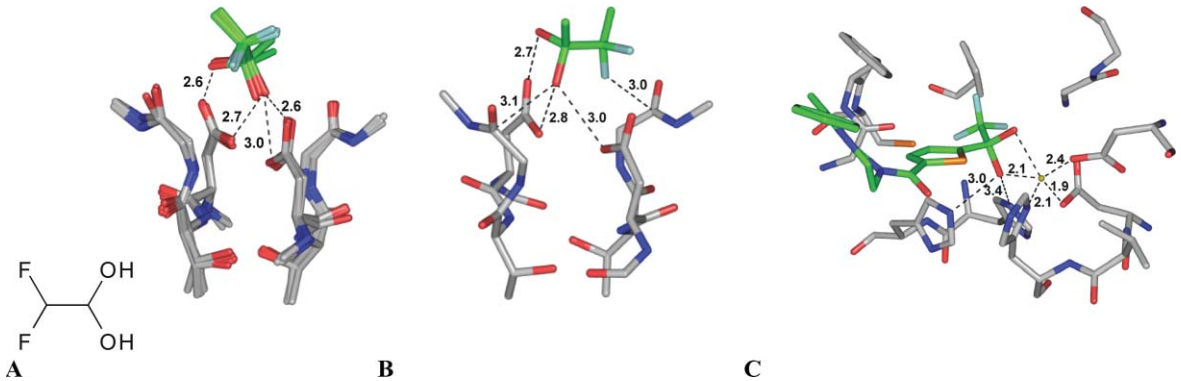


Fig. 1 Two different known binding modes for hydrated α,α-difluoroketones in aspartic proteases (A, B)[21–26] and a hydrated α,α,α-trifluoroketone[29] binding to the human HDAC4 catalytic domain (C). The distances in Fig. 1A are given for the ligand bound to the protein with the PDB code 1APV.[26] The PDB search for all binding patterns was based on the molecule represented in Fig. 1A. In this and the following figures, all distances are given in Å. Colour code: C atoms, grey (protein), green (ligand); O atoms, red; N atoms, blue; F atoms, light blue; S atoms, orange.

structures of aspartic proteases, inhibited by α,α-difluoroketone hydrates, two different binding motifs were found (Fig. 1).[21–26] Seven hydrated α,α-difluoroketones define a binding mode (Fig. 1A), in which the diol forms strong H-bonds to both Asp side chains. In this binding mode, the C–F bonds point towards the solvent-exposed area of the protein. A second binding mode is found for an HIV protease inhibitor (Fig. 1B), where the hydrated α,α-difluoroketone moiety is located between the Gly and Asp residues of the two Asp-Thr-Gly sequences.[25] In this geometry, the two OH moieties of the difluoroketone hydrate ligand engage again in H-bonding interactions with the Asp side chains, while one C–F and one C–O(H) unit undergo orthogonal dipolar interaction with the C=O moieties of the two Gly residues.[27] Computer modeling using MOLOC[28] suggested that the new cyclic difluoroketone hydrates described below could establish similar protein–ligand interactions.

Finally, for hydrated α,α,α-trifluoroketones, the PDB search revealed twelve structures. As an example, in the human histone deacetylase-4 (HDAC4) catalytic domain, the two OH groups of a hydrated α,α,α-trifluoroketone inhibitor ligate to a Zn(II) ion in the active site (Fig. 1C).[29] This example hints at a further field of applications for new cyclic α,α-difluoroketone hydrates, beyond aspartic protease inhibition.

Results and discussion

Synthesis of alicyclic α,α-difluoroketones

Compounds (±)-**1–4** are direct precursors of the desired hydrates and resulted from structure-based design using MOLOC (see Experimental). They comprise either a central cyclohexanone or cyclopentanone ring ("needle"), which is equipped with the difluoro-ketone as a warhead and a single amide unit, that can direct a 4-pentylphenyl group into the "flap-open" pocket and a 2-naphthyl substituent into the spacious S1/S3 pocket (Fig. 2).[7–9,30,31] The analogous piperidinium salts **5** and **6** were prepared for comparison. The developed synthetic route is illustrated in the main manuscript for the preparation of (±)-**1** and (±)-**3**, whereas the syntheses of (±)-**2**, (±)-**4** and **5**/**6** are included in the electronic supplementary information (ESI).†

In the synthesis of (±)-**1**, 2-cyclohexen-1-one (**7**) was oxidised with $Pb(OAc)_4$ to the acetate (±)-**8**, which was subsequently vinylated (vinylmagnesium bromide, CuI) in a Michael-addition to afford the two diastereoisomeric pairs of enantiomers (±)-**9** and (±)-**10** (Scheme 1). The constitution and relative configuration of (±)-**9** and (±)-**10** were proven by ^{1}H,^{1}H COSY and 1D-NOE NMR experiments as well as by the X-ray crystal structure solved for the 4-substituted α,α-difluorinated cyclohexan-1-ol (±)-**11** (see Scheme 2). Difluorination (DAST) of ketones (±)-**9** and (±)-**10** yielded (±)-**12** and (±)-**13**. The difluorinated building blocks (±)-**12** and (±)-**13** were oxidised ($RuCl_3$, $NaIO_4$)[32] to the corresponding carboxylic acids which were subsequently converted ($SOCl_2$) into the acyl chlorides (±)-**14** and (±)-**15**. Alternatively, (±)-**12** and (±)-**13** were oxidised (OsO_4, $NaIO_4$) to the corresponding aldehydes (±)-**16** and (±)-**17**, respectively.

The ligand fragment with the residues to fill the sub-pockets was constructed based on the commercial available reagents 2-naphthylacetic acid (**18**) and 4-pentylaniline (**19**). Therefore, **18** was chlorinated ($SOCl_2$) to the acyl chloride **20** and subsequent coupling with 4-pentylaniline (**19**) to give amide **21** (Scheme 2).

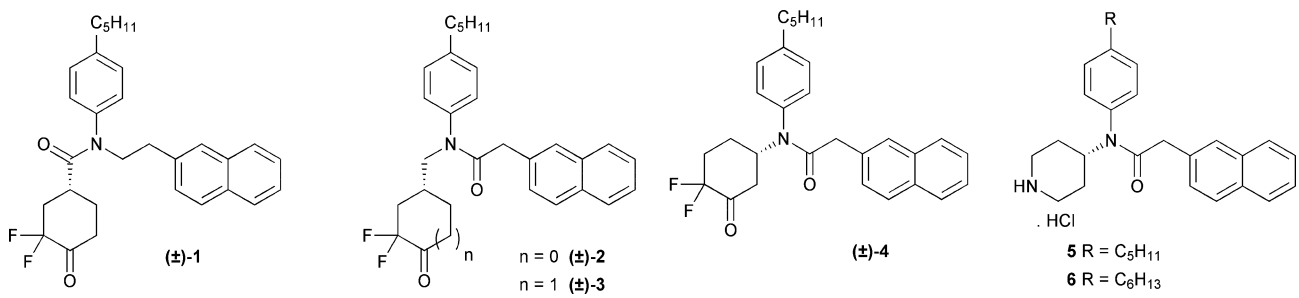
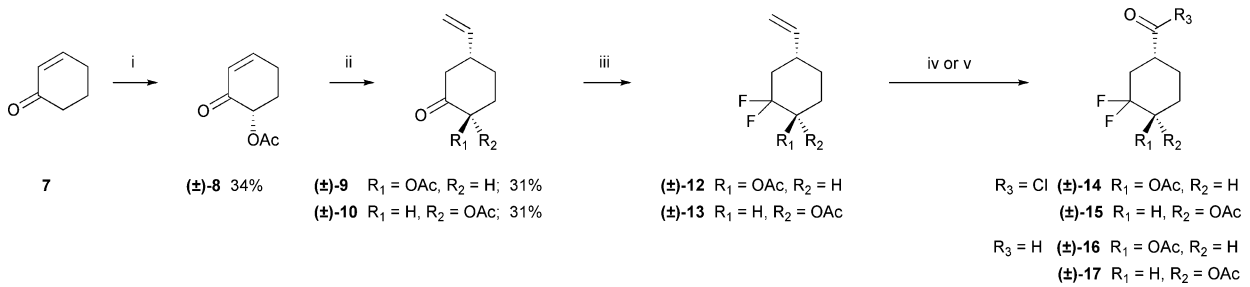
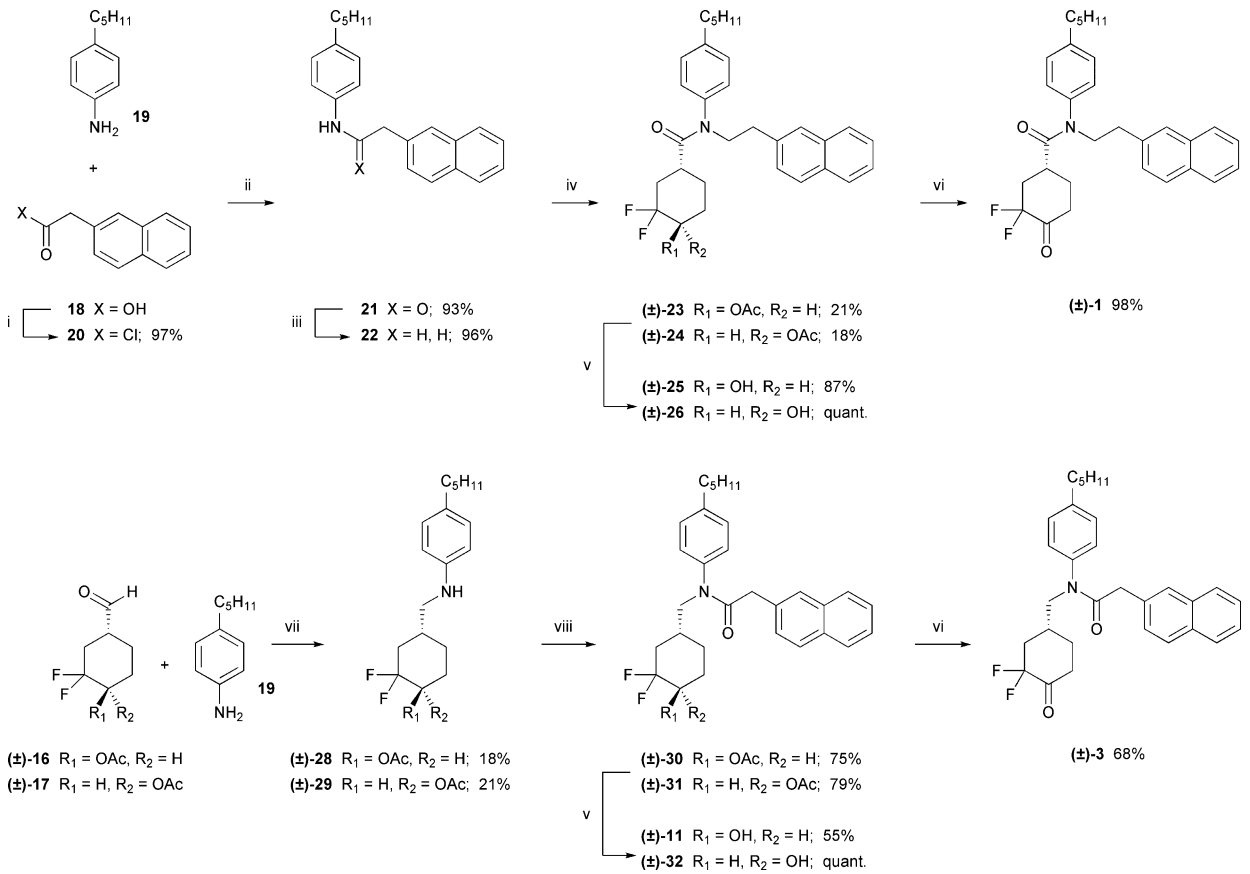


Fig. 2 α,α-Difluorinated cycloalkanones (±)-**1** to (±)-**4** which, in the hydrated form, are expected to bind to PMs, and reference inhibitors **5** and **6**.



Scheme 1 Synthesis of the α,α-difluorinated building blocks (±)-**14** to (±)-**17**. *Reagents and conditions:* (i) $Pb(OAc)_4$, benzene, reflux, 18 h; (ii) vinylmagnesium bromide, CuI, TMEDA, TMSCl, THF, −78 °C, 3 h; (iii) DAST, CH_2Cl_2, reflux, 22 h; (iv) $RuCl_3$, $NaIO_4$, $CH_3CN/H_2O/CCl_4$ 2:3:2, r.t., 20 h; then $SOCl_2$, CH_2Cl_2, r.t., 5 h; (v) OsO_4, $NaIO_4$, dioxane/H_2O 10:1, r.t., 12 h. In steps (iii), (iv) and (v), only crude products were isolated. TMEDA = *N,N,N′,N′*-tetramethylethylenediamine, TMS = trimethylsilyl, DAST = diethylaminosulfur trifluoride.



Scheme 2 Synthesis of difluoroketones (±)-**1** and (±)-**3**. *Reagents and conditions:* (i) $SOCl_2$, CH_2Cl_2, r.t., 16 h; (ii) Et_3N, THF, r.t., 1 h; (iii) BH_3·THF, THF, r.t., 3 h; (iv) (±)-**14** or (±)-**15**, DIPEA, CH_2Cl_2, r.t., 90 min; (v) K_2CO_3, H_2O, MeOH, CH_2Cl_2, r.t., 3 h; (vi) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 7 h, then 4 Å molecular sieves, CH_2Cl_2, 3 h; (vii) $NaBH(OAc)_3$, CH_2Cl_2, r.t., 3.5 h; (viii) 2-naphthylacetic acid chloride (**20**), DIPEA, CH_2Cl_2, r.t., 24 h. DIPEA = diisopropylethylamine.

Reduction (BH_3·THF) of **21** yielded aniline **22**, which was coupled (DIPEA) with the acyl chlorides (±)-**14** and (±)-**15** to give amides (±)-**23** and (±)-**24**, respectively. Saponification (K_2CO_3, MeOH/H_2O) yielded (±)-**25** and (±)-**26**, and oxidation of (±)-**26** under mild conditions (Dess–Martin periodinane) gave ketone (±)-**1**. It is worth mentioning that the isolated crude product consisted of a mixture of ketone (±)-**1** and its hydrated derivative (±)-**27** (Fig. 3). Stirring over molecular sieves (4 Å) pushed the equilibrium completely to the side of ketone (±)-**1**.

Aldehydes (±)-**16** and (±)-**17** were coupled with 4-pentylaniline (**19**) in a reductive amination ($NaBH(OAc)_3$) to give amines (±)-**28** and (±)-**29**, respectively (Scheme 2). The latter were coupled with acyl halide **20** to afford amides (±)-**30** and (±)-**31**, which were saponified to yield (±)-**11** and (±)-**32**, respectively. Subsequent oxidation of (±)-**32** gave (±)-**3**.

The cyclopentanone-based inhibitor (±)-**2** and the more rigid α,α-difluoroketone (±)-**4** were prepared in a similar way, whereas piperidines **5** and **6** were obtained in three steps starting from commercial reagents (see ESI).†

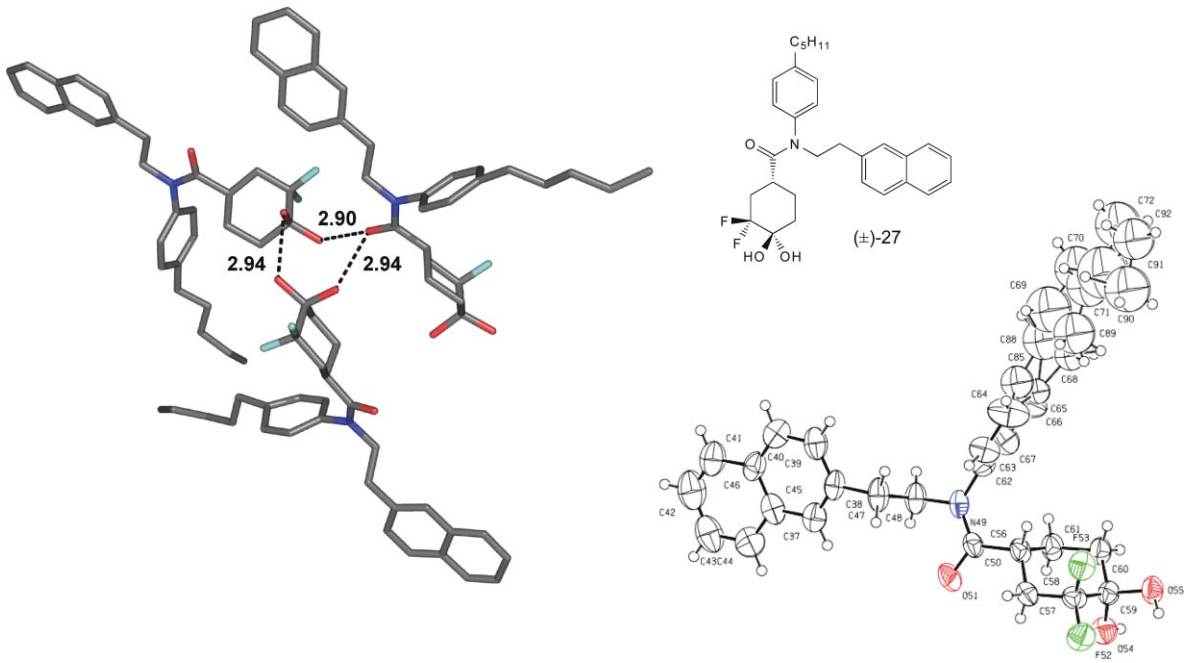


Fig. 3 X-Ray crystal structure of the hydrated α,α-difluoroketone (±)-**27** with two symmetry independent molecules. Selected torsional angles (°): F53–C58–C59–O55 = 50 (53, molecule omitted in plot), F53–C58–C59–O54 = 170 (174), F52–C58–C59–O54 = 55 (61), F52–C58–C59–O55 = –65 (–60). Left: H-bond pattern. The hydrate moieties participate both as H-bond donor and acceptor in a hydrogen-bond network. Lower right: ORTEP plot of one molecule showing the disorder of the pentyl substituent. Numbering is arbitrary. Atomic displacement parameters obtained are drawn at the 50% probability level.

Hydration of α,α-difluoroketones in the solid and liquid state

It was not possible to develop a general procedure for the isolation of difluoroketones (±)-**1**–**4**, in their biologically active hydrated form (see below). A search for α,α-difluoroketones in the Cambridge Structural Database (CSD) confirms this observation.[33] A total of 316 keto structures was found, which is substantially higher than the number of structures identified in a similar search for α,α-difluorohydrates (see below). The search showed that even for perfluorinated ketones, a fluorinated ketone is preferred over the hydrated form in the solid state. Nevertheless, out of the isolated mixture of ketone (±)-**1** and its hydrated derivative (±)-**27**, it was possible to grow crystals suitable for X-ray analysis consisting exclusively of hydrate (±)-**27**. The mixture (±)-**1**/(±)-**27** was simply allowed to stand under air at room temperature and after three days, nearly all of the mixture had formed crystals. The subsequent X-ray crystal structure analysis revealed that the crystals consisted only of hydrated ketone (±)-**27**. It is believed that due to the highly electrophilic nature of its carbonyl carbon, ketone (±)-**1** readily reacts with moisture from air forming (±)-**27** (Fig. 3).

In the crystal, hydrate (±)-**27** forms a H-bonded network involving two neighbouring molecules. The amide C=O of one molecule acts as H-bond acceptor for O–H residues of two neighbouring hydrates, which also interact intermolecularly *via* an additional O–H···O–H-bond. The analysis of the torsional angles (Fig. 3 caption) reveals that the vicinal F and OH substituents are staggered in the cyclohexane chair. A search for α,α-difluorohydrates was performed in the X-ray (CSD).[33] A total of 27 structures were found, of which 24 are α,α,α-trifluorohydrates. This result could be expected, since the ready synthetic availability of α,α,α-trifluorinated carbonyl groups is well documented in the literature. One structure is based on a hydrated α,α-difluorinated aldehyde,[34] and two structures are based on 2,2-difluorocyclohexane-1,3-diones,[35] in which one of the keto functions is hydrated. In a 2,2,4,4,6,6-hexafluorocyclohexane-1,1,3,3,5,5-hexaol, a staggered alignment of the F and OH substituents was observed.[35] In the crystal lattice, this structure forms H-bond mediated clusters, comparable to the ones seen in the crystals of (±)-**27**. Generally, hydrates, which are present in solid-state structures, display water-like behaviour in terms of their intermolecular H-bonding interactions.[36]

A focus of this work was the application of a hydrated α,α-difluoroketone in a biological system. The molecular recognition capacity of a ketone is very different from a *gem*-diol: whereas the keto function can only act as a H-bond acceptor, the *gem*-diol can act as a double H-bond donor and/or acceptor. A considerable amount of keto form could lead to possible repulsive interactions with the catalytic dyad in aspartic proteases, since at least one of the two Asp side chains is deprotonated. The keto–hydrate equilibrium also depends on the electrophilicity of the carbonyl carbon and the possibility to solvate the resulting *gem*-diol. Formaldehyde exists to 99.99% in the hydrated form in water at 20 °C. In comparison, significantly less acetaldehyde is converted to its hydrate (58%) and for acetone, the amount of hydrated species is negligible.[37] The introduction of highly electronegative F-substituents pulls electron density from the carbonyl functionality and significantly increases the electrophilicity of the C=O carbon. Therefore, the equilibrium constant K_{hydr} = [hydrate]/[ketone] in water for the hydrate/ketone equilibrium of

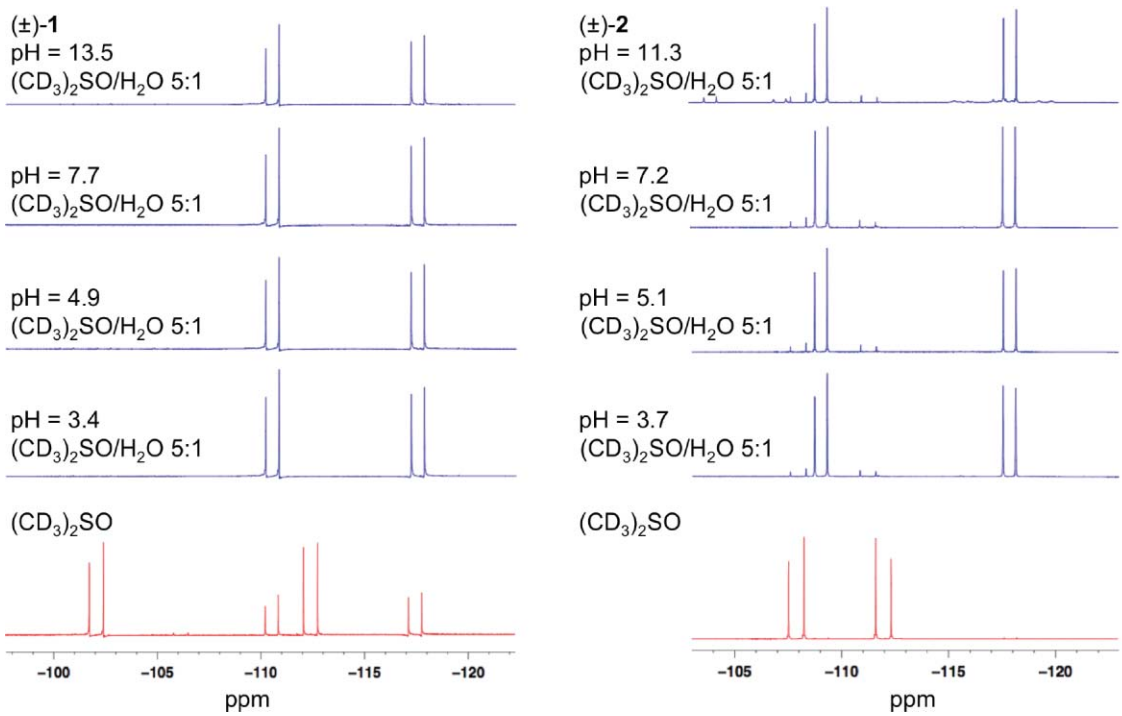


Fig. 4 ^{19}F NMR spectra (376 MHz, 300 K) of the mixture of (±)-**1**/(±)-**27** (left) and (±)-**2** (right) in $(CD_3)_2SO$ and in the solvent mixture $(CD_3)_2SO/H_2O$ 5:1 at various pH-values. pH values were measured with an NMR pH electrode.

acetone is $K_{hydr} = 0.0014$, of α-fluoroacetone $K_{hydr} = 0.17$ and of α,α,α-trifluoroacetone $K_{hydr} = 32.36$.[38] H-bond networks are the dominating intermolecular interactions in the corresponding solid-state structures, and pertinent overviews are given in the cited literature.[39,40]

We conducted a comparative study to analyse the degree of hydrate formation of the cyclohexyl- and cyclopentyl-based α,α-difluoroketones (±)-**1** and (±)-**2** in solution. ^{19}F NMR spectroscopy was chosen to determine the keto/hydrate ratio. Sufficient solubilisation of the compounds for the NMR investigations required the addition of an excess of deuterated dimethyl sulfoxide ($(CD_3)_2SO$), and the investigation was conducted in $(CD_3)_2SO/H_2O$ 5:1 while the pH of the solution was adjusted by addition of aqueous HCl and NaOH solutions.

A mixture of cyclohexyl-based (±)-**1** and (±)-**27** was dissolved in pure $(CD_3)_2SO$ and the ^{19}F NMR spectra recorded at 300 K, displaying a mixture of keto (downfield shifted signals at −102.1 and −112.4 ppm) and hydrate form (upfield shifted signals at −110.5 and −117.4 ppm) (Fig. 4). When water was added to this mixture, the hydrated species (±)-**27** formed nearly quantitatively, yielding on average a keto/hydrate ratio (signal integration) of 0.2:99.8 at pH-values between pH 3.5 and 13.5. The pure cyclopentyl-based α,α-difluoroketone (±)-**2** is not hydrated in pure $(CD_3)_2SO$, and its ^{19}F NMR resonances appear at −110.6 and −117.6 ppm. Upon addition of aqueous solutions in the pH-range between 3.7 and 7.2 to give a solvent mixture $(CD_3)_2SO/H_2O$ of 5:1, a keto/hydrate ratio of 4.4:95.6 was measured, starting from (±)-**2**. Thus, the equilibrium constant for hydration in $(CD_3)_2SO/H_2O$ at 300 K is substantially larger for (±)-**1** ($K_{hydr} = 499$, $\Delta G_{hydr} = -15.5$ kJ/mol) than for (±)-**2** ($K_{hydr} = 22$, $\Delta G_{hydr} = -7.7$ kJ/mol). Note that in basic conditions (pH = 11.3), the cyclopentyl-based α,α-difluoroketone (±)-**2** shows additional signals in the ^{19}F NMR spectrum (Fig. 4), which could possibly originate from by-products formed by enolate chemistry.

The ^{19}F NMR spectra clearly reveal that (±)-**1** and (±)-**2** both prefer to be hydrated in an aqueous environment, an absolute necessity for biological activity against the PMs. This preference is much more pronounced in the case of difluorocyclohexanone (±)-**1** than in the case of difluorocyclopentanone (±)-**2**. The change from sp^2 ((±)-**1**) to sp^3 (in (±)-**27**) hybridisation releases torsional strain (Pitzer strain) in the six-membered ring, whereas this strain is increased in the five-membered ring upon changing from (±)-**2** to the corresponding hydrate. In particular, repulsive vicinal F···O interactions might account for the reduced propensity of ketone (±)-**2** to convert into its hydrated form. Vicinal F and OH substituents in a five-membered ring are poorly staggered or even eclipsed, which leads to strong repulsion between lone pairs.[41] In contrast, the F and OH substituents in a cyclohexane chair are well staggered as seen in the X-ray crystal structure analysis of the difluorohydrate (±)-**27** (Fig. 3).

Biological results

Biological affinities of the inhibitors for the respective target enzymes (Table 1) were measured with a fluorescence resonance energy transfer (FRET) assay (see Experimental). The following conclusions can be drawn: (i) α,α-difluorocyclohexanone-based inhibitors (±)-**1** and (±)-**3** show IC_{50} values for PM II down to the single digit micromolar range. Their affinities towards PM IV are substantially weaker compared with PM II. Compounds (±)-**1** and (±)-**3** probably bind in their hydrated form (see above) and are three to four times more active than the corresponding monohydroxy derivates (±)-**25**/(±)-**26** or (±)-**11**/(±)-**32**. (ii) Inhibitors in which the carbonyl group of the amide is oriented towards the S1/S3 pocket

Table 1 Binding affinities against PM II and IV as well as the human cathepsins Cath D and Cath E. Compounds in the Table are arranged to facilitate comparison between the difluorinated cycloalkanols (*e.g.* (±)-**25**/(±)-**26**) and the hydrated cycloalkanones (*e.g.* (±)-**1**). n.d.: not done

Compound (IC_{50} in μM)	PM II	PM IV	Cath D	Cath E
(±)-**25**	43	>100	>100	>100
(±)-**26**	38	>100	>100	>100
(±)-**1**	10	73	>100	>100
(±)-**32**	16	>100	>100	>100
(±)-**11**	19	>100	>100	>100
(±)-**3**	7	57	87	87
(±)-**33**	>100	>100	>100	>100
(±)-**34**	>100	>100	>100	>100
(±)-**2**	>100	>100	>100	>100
(±)-**35**	24	>100	>100	>100
(±)-**36**	20	>100	>100	>100
(±)-**4**	24	65	>100	>100
5	19	n.d.	n.d.	n.d.
6	13	n.d.	n.d.	n.d.

((±)-**3**, (±)-**11** and (±)-**32**) are slightly more active compared to (±)-**1**, (±)-**25** and (±)-**26** with the amide *N*-atom pointing towards the S1/S3 pocket. Computer modeling suggests (Fig. 5) that the amide C=O group in the complexes of the latter is solvent-exposed, while it could engage in a H-bond with Tyr77 in the complexes of the former (not shown). (iii) Interestingly, cyclopentyl-based inhibitors (±)-**2**, (±)-**33** and (±)-**34** display no activity towards the PMs within the sensitivity range of the enzyme assay (up to IC_{50} values of 100 μM). We assume that the higher conformational flexibility of the cyclopentane ring in conjunction with its rapid puckering motion, is responsible for this finding. Also, the conversion of (±)-**2** to its hydrate might be reduced as compared to (±)-**1**. (iv) A comparison of (hydrated) difluorocyclohexanone (±)-**4** with (protonated) piperidinium ligands **5** and **6** shows similar activities for the two classes of compounds, suggesting that suitably functionalized hydrated α,α-difluorocyclohexanones could serve as new, alternative needles to address the catalytic Asp dyad in various aspartic proteases. (v) Finally, activities of the new ligands against the human cathepsins (Cath) D and E are low, which is in agreement with previous proposals[7] that these aspartic proteases do not feature a "flap-open" pocket to accommodate the *n*-alkyl substituent of the ligands.

Conclusions

The first alicyclic α,α-difluoroketones have been synthesised as potential inhibitors for PMs. They were expected to bind in their hydrated form to the catalytic Asp dyad of the enzymes. All α,α-difluoroketones were isolated in their keto form from organic solvents but became readily hydrated in aqueous solution or even in the presence of atmospheric moisture. The X-ray crystal structure proved the presence of hydrated α,α-difluorocyclohexanone (±)-**27** and further demonstrated the potency of the OH groups of the hydrate moiety to act as H-bond donors and acceptors in H-bonding networks. A cyclohexyl-based α,α-difluoroketone was found by ^{19}F NMR spectroscopy to be nearly completely hydrated in an aqueous environment (K_{hydr} = 499 in $(CD_3)_2SO/H_2O$ 5:1, keto/hydrate ratio: 0.2:99.8) whereas a significant amount of the keto form of an analogous cyclopentyl-based derivative (K_{hydr} = 22, keto/hydrate ratio: 4.4:95.6) remained present under the same conditions. This was explained by the differences in Pitzer strain and in particular repulsive F···O interactions in the two difluorinated cycloalkanones and their corresponding hydrates. Biological assays showed that some hydrated α,α-difluorocyclohexanones had IC_{50}-values reaching single digit micromolar values against PM II, whereas the corresponding cyclopentanone derivative did not bind within the sensitivity limits (100 μM) of the assay.

Experimental

General details

Solvents and reagents were reagent-grade, purchased from commercial suppliers, and used without further purification unless otherwise stated. THF was freshly distilled from sodium benzophenone, CH_2Cl_2 from CaH_2 and toluene from sodium. If not mentioned otherwise, all products were dried under high vacuum (10^{-2} Torr) before analytical characterisation. Column chromatography (CC) was conducted on silica gel (230–400 mesh, 0.040–0.063 mm) from Fluka. Analytical thin layer

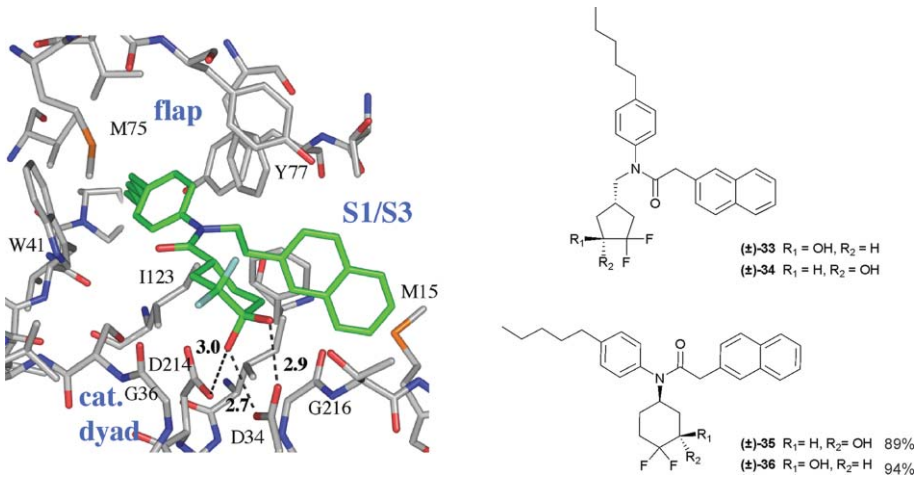


Fig. 5 Left: A possible binding geometry for inhibitor (±)-**1** modeled into the active site of PM II (PDB code: 1IGX) generated with the modeling software MOLOC.[28] Right: The α,α-difluoroalcohols (±)-**33**–**36**.

chromatography (TLC) was conducted on silica gel 60-F_{254} nm (on glass, Merck). Plates were visualised by UV light at 245 nm and staining with a solution of $KMnO_4$ (1.5 g), K_2CO_3 (10 g), 5% NaOH (2.5 cm^3) in H_2O (150 cm^3). Melting points (mp) were determined using a Büchi-510 apparatus and are uncorrected. IR Spectra: Perkin Elmer Spectrum BX FTIR System spectrometer (ATR-unit, Attenuated Total Reflection, Golden Gate). NMR spectra (^1H, ^{13}C, ^{19}F, ^1H-^1H COSY): Varian Gemini-300, Bruker, AV-400 and DRX-400; spectra were recorded at 25 °C using the solvent peak as an internal reference. Coupling constants (J) are given in Hz. The resonance multiplicity is described as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The exchangeable OH signals are not always observed in ^1H NMR spectra. Mass spectra were recorded on a Varian-IonSpec-MALDI-FT-ICR (MALDI, with 2,5-dihydroxybenzoic acid as the Matrix), on a Waters-Micromass-AutoSpec-Ultima (EI, 70 eV) and a Varian IonSpec ESI-FT-ICR. If the molecular ion [M^+] is the parent peak, only its m/z-value is reported. If a fragment is the parent peak, the relative intensity of the molecular ion, as well as the fragment with 100% intensity will be given. For MALDI spectra, the $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ peaks are reported, if they occur in the spectra. The nomenclature was generated with the computer program ACD-Name (ACD/Labs).

FRET assay for the determination of IC_{50} values[9]

The proteolytic activity of the particular enzyme was tested in a FRET assay. M-2120 from Bachem was used as the substrate. The enzyme, with an approximative concentration of 1 nM, was incubated with the substrate (concentration ≈ 1 μM) at 37 °C in the presence of a sodium acetate solution (50 mM), at pH 5, 12.5% (V/V) glycerol, 0.1% (V/V) bovine-serum-albumin and 10% $(CH_3)_2SO$. The enzyme activity was determined by the rate of conversion of the substrate, by plotting the corresponding signal of the increasing fluorescence. The fluorescence was analyzed with FluoroStar Galaxy from BMG, and the excitation and emission filters 355 and 520 nm, respectively, were used. The test compounds were dissolved and diluted with 100% $(CH_3)_2SO$. The biological activity is expressed by the IC_{50}-value.

Molecular modeling

Potential inhibitors were manually docked within the known structure of PM II, co-crystallized in complex with an inhibitor based on a piperidinium needle and with a "flap-open" pocket (pdb code: 2IGX).[8] The enzyme structure without the piperidinium ligand was fixed and the energy of the system minimized using the MAB force field as implemented in the computer program MOLOC.[28] Evaluation of different binding conformations of the inhibitors was based on (i) avoidance of unfavourable steric contacts, (ii) formation of favourable H-bonding contacts and (iii) optimal filling of the space within binding pockets, by establishing the maximal number of attractive van-der-Waals contacts between enzyme and ligand. For the chiral inhibitors (±)-**1–4** and their corresponding precursors, the *R*-enantiomer fits best into the active site according to the modeling. For comparative reasons, all modelling was also performed starting from two similar co-crystal structures (pdb code: 2IGY[8] and 2BJU[9]), following the same procedure as described.

General procedure A for the α-oxidation of ketones

The ketone (1.0 eq.) was dissolved in degassed toluene under Ar at r.t., and lead tetraacetate (1.75 eq.) was added. The mixture was stirred under reflux. The suspension was filtrated over Celite, and the resulting organic phase was concentrated *in vacuo.* Purification by CC (SiO_2; EtOAc/pentane) afforded the product.

Procedure B for the Michael addition of vinylmagnesium bromide

Copper iodide (0.06 eq.) was suspended in THF at 0 °C, and TMEDA (1.2 eq.) was added. The solution was stirred for 5 min at 0 °C, cooled down to −78 °C, and vinylmagnesium bromide (1 M in THF, 1.1 eq.) was added dropwise for 30 min. The mixture was stirred for 20 min at −78 °C, and TMSCl (1.2 eq.) and the ketone (1.0 eq.), dissolved in THF, were added, and the resulting solution was stirred at −78 °C. The mixture was treated with saturated aqueous NH_4Cl solution and warmed up to r.t. The mixture was extracted with Et_2O, and the combined organic phases were washed with 1 M aqueous HCl solution, followed by saturated aqueous NaCl solution. The organic phase was dried over $MgSO_4$, filtrated and concentrated *in vacuo.* Purification by CC (SiO_2; EtOAc/pentane) afforded the product.

General procedure C for the difluorination of a ketone with DAST

The ketone (1.0 eq.) was dissolved in CH_2Cl_2 under Ar at r.t. and DAST (2.0 eq.) was added to the stirred solution. The mixture was stirred under reflux and subsequently cooled down to r.t. The solution was treated with a second portion of DAST (2.0 eq.), and stirring under reflux was continued. The mixture was poured into a saturated aqueous $NaHCO_3$ solution and extracted three times with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtrated, and the solvent was concentrated *in vacuo.* Purification by CC (SiO_2; EtOAc/pentane) afforded the product.

Procedure D for the conversion of an acid to the acid chloride

Acid (1.0 eq.) was dissolved in CH_2Cl_2 and the solution was cooled down to 0 °C. To the stirring solution, $SOCl_2$ (5.0 eq.) was added slowly and the mixture was stirred at r.t. The organic phase was concentrated *in vacuo* yielding the crude product.

General procedure E for the coupling of an acyl chloride with an amine

The amine (1.0 eq.) was dissolved in THF under Ar at r.t., and acyl chloride (1.0 eq.) was added. DIPEA (1.2 eq.) was added and the mixture stirred at r.t. The organic phase was concentrated *in vacuo* and the resulting crude product dissolved in EtOAc and washed with water. The organic phase was dried over $MgSO_4$, filtrated and concentrated *in vacuo.* Purification by CC (SiO_2; EtOAc/pentane) yielded the product.

Procedure F for the oxidation of a terminal olefin to an acid

Olefin (1.0 eq.) was dissolved in a solvent mixture of $MeCN/H_2O/CCl_4$ (2:3:2). $RuCl_3$ (0.01 eq.) and $NaIO_4$ (4.0 eq.) were added, and the mixture was stirred at r.t. The mixture was treated with water and the aqueous phase extracted with CH_2Cl_2.

The combined organic phases were dried over $MgSO_4$, filtrated and concentrated *in vacuo* yielding the crude acid.

General procedure G for the saponification of an acetyl ester

The acetate (1.0 eq.) was dissolved in CH_2Cl_2 under Ar at r.t., and an aqueous K_2CO_3/MeOH solution (1.0 g K_2CO_3 dissolved in 30 cm^3 H_2O and 115 cm^3 MeOH) was added. The mixture was stirred at r.t., and a saturated aqueous NH_4Cl solution was added. The water phase was extracted with CH_2Cl_2, and the combined organic phases were dried over $MgSO_4$, filtrated and concentrated *in vacuo.* Purification by CC (SiO_2; EtOAc/pentane) afforded the product.

General procedure H for the oxidation of an alcohol to a ketone

The secondary alcohol (1.0 eq.) was dissolved in CH_2Cl_2 under Ar at r.t., and a 15% Dess–Martin periodinane solution in CH_2Cl_2 (2.0 eq.) was added at 0 °C. The mixture was stirred at r.t. and subsequently treated with a saturated aqueous $NaHCO_3$ solution and a 10% aqueous NaS_2O_3 solution. After stirring for 30 min at r.t., the aqueous phase was extracted with CH_2Cl_2 and the combined organic phase dried over $MgSO_4$, filtrated and concentrated *in vacuo.* The crude product was dissolved in dry CH_2Cl_2, molecular sieves (4 Å, 20 mg) were added, and the mixture was stirred at r.t. for 3 h. Purification by CC (SiO_2; EtOAc/pentane) afforded the product.

(1*RS*)-2-Oxocyclohex-3-en-1-yl acetate ((±)-8).[42] Using general procedure A, cyclohexenone (**7**) (25.00 g, 260 mmol) and $Pb(OAc)_4$ (201.80 g, 455 mmol) in toluene (300.0 cm^3) for 18 h, followed by CC purification (SiO_2; EtOAc/pentane 1:4), afforded (±)-**8** (13.7 g, 34%) as a pale yellow oil. δ_H(300 MHz; $CDCl_3$) 2.09–2.18 (1 H, m), 2.18 (3 H, s), 2.19–2.31 (1 H, m), 2.54–2.61 (2 H, m), 5.37 (1 H, dd, *J* 5.5 and 13.6), 6.07 (1 H, ddd, *J* 1.6, 2.5 and 7.5), 6.93–6.99 (1 H, m).

(1*RS*,4*SR*)-4-Ethenyl-2-oxocyclohexyl acetate ((±)-9) and (1*RS*,4*RS*)-4-ethenyl-2-oxocyclohexyl acetate ((±)-10). Using general procedure B, CuI (148 mg, 0.78 mmol), TMEDA (2.349 cm^3, 1.809 g, 15.57 mmol), vinylmagnesium bromide (1 M solution in THF, 20.387 cm^3, 20.39 mmol), TMSCl (1.99 cm^3, 1.691 g, 15.57 mmol) and ketone (±)-**8** (2.000 g, 12.94 mmol, 1.0 eq., dissolved in THF (50 cm^3)) in THF (100 cm^3) for 3 h, followed by CC purification (SiO_2; EtOAc/pentane 1:6), afforded (±)-**9** (743 mg, 31%) as a white solid and (±)-**10** (737 mg, 31%) as a yellow oil in the given elution order. (±)-**9**: mp 40 °C; Found: C, 66.13; H, 7.83. $C_{10}H_{14}O_3$ requires C, 65.92; H, 7.74%; ν_{max}(solid)/cm^{-1} 2938, 1745, 1725, 1450, 1375, 1232, 1083, 1049, 896; δ_H(300 MHz; $CDCl_3$) 1.59–1.74 (1 H, m), 1.73–1.87 (1 H, m), 2.00–2.15 (1 H, m), 2.16 (3 H, s), 2.27–2.35 (1 H, m), 2.29–2.35 (1 H, m), 2.41–2.46 (1 H, m), 2.52–2.59 (1 H, m), 5.01–5.08 (2 H, m), 5.17 (1 H, dd, *J* 6.5, 12.5), 5.72–5.84 (1 H, m); δ_C(75 MHz, $CDCl_3$) 20.8, 29.9, 31.3, 43.1, 45.9, 76.1, 114.1, 140.1, 169.8, 203.1; *m*/*z* (EI) 182.0960 (3%, [M^+]. $C_{10}H_{14}O_3^+$ requires 182.0943), 140.0834 (20), 122.0727 (16), 43.0178 (100). (±)-**10**: Found: C, 66.20; H, 7.85. $C_{10}H_{14}O_3$ requires C, 65.92; H, 7.74%; ν_{max}(solid)/cm^{-1} 2945, 2361, 1747 (CO), 1724 (CO), 1640, 1375, 1233, 1070, 921; δ_H(300 MHz; $CDCl_3$) 1.88–1.93 (1 H, m), 1.95–2.11 (3 H, m), 2.14 (3 H, s), 2.59–2.62 (2 H, m), 2.90–2.94 (1 H, m), 5.04–5.14 (2 H, m), 5.12–5.16 (1 H, m), 5.69–5.80 (1 H, m); δ_C(75 MHz, $CDCl_3$) 20.9, 28.0, 28.4, 39.7, 43.6, 76.3, 116.3, 138.9, 169.8, 203.8; *m*/*z* (EI) 182.0953 (2%, [M^+]. $C_{10}H_{14}O_3^+$ requires 182.0943), 140.0831 (7), 122.0727 (8), 43.0248 (100).

2-Naphthylacetic acid chloride (20).[43] Using general procedure D, 2-(naphthalen-2-yl)acetic acid (**18**) (1.000 g, 5.37 mmol) and $SOCl_2$ (1.95 cm^3, 3.194 g, 26.85 mmol) in CH_2Cl_2 (2.0 cm^3) for 16 h gave the crude product **20** (1.099 g, 97%) as a brown solid. mp 62 °C (Lit. 65 °C[43]); δ_H(300 MHz; $CDCl_3$) 4.31 (2 H, s), 7.37 (1 H, dd, *J* 1.9, 8.4), 7.48–7.75 (2 H, m), 7.82 (1 H, s), 7.82–7.88 (3 H, m).

2-(Naphthalen-2-yl)-*N*-(4-pentylphenyl)acetamide (21). Using general procedure E, 4-pentylaniline **19** (3.99 g, 4.34 cm^3, 24.43 mmol), acyl chloride **20** (5.00 g, 24.43 mmol) and Et_3N (3.41 cm^3, 2.41 g, 24.43 mmol) in THF (50 cm^3) for 1 h, followed by CC (SiO_2; EtOAc/pentane 1:5), afforded **21** (8.10 g, 93%) as a white solid. mp 144 °C; Found: C, 83.06; H, 7.44. $C_{23}H_{25}NO$ requires C, 83.35; H, 7.60%; ν_{max}(solid)/cm^{-1} 3245, 3117, 3057, 2924, 2866, 1650 (CO), 1599, 1538, 1410, 1259, 1165, 1124, 952, 896, 819, 738; δ_H(300 MHz; $CDCl_3$) 0.86 (3 H, t, *J* 6.8), 1.25–1.29 (4 H, m), 1.53–1.56 (2 H, m), 2.52 (2 H, t, *J* 7.8), 3.90 (2 H, s), 7.00 (1 H, s), 7.07, 7.28 (4 H, AA′BB′, *J* 8.5), 7.44 (1 H, dd, *J* 2.4, 8.4), 7.50–7.53 (2 H, m), 7.80 (1 H, s), 7.82–7.90 (3 H, m); δ_C(75 MHz, $CDCl_3$) 14.2, 22.7, 31.3, 31.5, 35.4, 45.0, 120.0, 126.1, 126.4, 127.2, 127.6, 127.7, 128.3, 128.7, 128.9, 132.0, 132.5, 133.5, 135.2, 139.1, 169.0; *m*/*z* (EI) 331.1937 (56%, [M+H]$^+$. $C_{23}H_{26}NO^+$ requires 331.1931), 106.0643 (100).

***N*-[2-(Naphthalen-2-yl)ethyl]-4-pentylaniline (22).** Amide **21** (7.14 g, 21.55 mmol) was dissolved in THF (40 cm^3), and BH_3·THF (1 M solution in THF, 6.47 cm^3, 64.65 mmol) was added at r.t. to the stirred solution. The solution was stirred at reflux for 3 h and subsequently cooled down to r.t. Saturated aqueous $NaHCO_3$ solution (60 cm^3) was added and aqueous phase extracted with CH_2Cl_2 (3 × 50 cm^3). The combined organic phases were dried over $MgSO_4$, filtrated and concentrated *in vacuo.* Purification by CC (SiO_2; EtOAc/pentane 1:19) afforded **22** (6.57 g, 96%) as a white solid. mp 63 °C; ν_{max}(solid)/cm^{-1} 3374, 3055, 2954, 2923, 2854, 1612, 1519, 1480, 1318, 1282, 1242, 1124, 899, 820, 743; δ_H(300 MHz; $CDCl_3$) 0.90 (3 H, t, *J* 6.7), 1.29–1.38 (4 H, m), 1.53–1.63 (2 H, m), 2.51 (2 H, t, *J* 7.8), 3.09 (2 H, t, *J* 7.0), 3.49 (2 H, t, *J* 6.8), 3.59 (1 H, br s), 6.59, 7.02 (4 H, AA′BB′, *J* 8.4), 7.38 (1 H, dd, *J* 1.5, 6.9), 7.48 (2 H, m), 7.68 (1 H, s), 7.83 (3 H, m); δ_C(75 MHz, $CDCl_3$) 14.2, 22.7, 31.6, 35.1, 35.8, 45.3, 113.1, 125.3, 126.0, 127.0, 127.1, 127.4, 127.5, 128.1, 129.0, 132.0, 132.1, 133.4, 136.7, 145.7; *m*/*z* (MALDI-HRMS) 318.2216 (100%, [M+H]$^+$. $C_{23}H_{28}N^+$ requires 318.2216).

(1*RS*,4*RS*)-2,2-Difluoro-4-{[2-(2-naphthyl)ethyl](4-pentyl-phenyl)carbamoyl}cyclohexyl acetate ((±)-23). Using general procedure C, ketone (±)-**9** (300 mg, 1.65 mmol) and DAST (880 mm^3, 1.062 g, 6.58 mmol) in CH_2Cl_2 (8 cm^3) for 22 h, followed by CC purification (SiO_2; EtOAc/pentane 1:9) yielded olefin (±)-**12**.

Using general procedure F, olefin (±)-**12** (200 mg, 0.98 mmol), $RuCl_3$ (2.2 mg, 0.01 mmol) and $NaIO_4$ (838 mg, 3.92 mmol) in MeCN/H_2O/CCl_4 (2:3:2) (7 cm^3) for 20 h yielded the crude carboxylic acid.

Using general procedure D, the crude acid and $SOCl_2$ (563 mm^3, 922 mg, 2.90 mmol) in CH_2Cl_2 (1 cm^3) for 5 h yielded the crude acyl chloride (±)-**14**.

Using general procedure E, crude acyl chloride (±)-**14**, amine **22** (100 mg, 0.42 mmol) and DIPEA (72 mm^3, 54 mg, 0.42 mmol) in CH_2Cl_2 (2.5 cm^3) for 90 min, followed by CC purification (SiO_2; EtOAc/pentane 1:5), afforded (±)-**23** (114 mg, 21%) as a colourless oil. Found: C, 73.41; H, 7.20; N, 2.68; F, 7.00. $C_{32}H_{37}F_2NO_3$ requires C, 73.68; H, 7.15; N, 5.69; F, 7.28%; ν_{max}(solid)/cm^{-1} 2931, 2858, 1740 (CO), 1652 (CO), 1510, 1409, 1368, 1231, 1178, 1095, 1061, 954, 817, 744; δ_H(300 MHz; $CDCl_3$) 0.93 (3 H, t, *J* 6.9), 1.27–1.42 (5 H, m), 1.56–1.67 (2 H, m), 1.67–1.73 (2 H, m), 1.90–1.98 (1 H, m), 2.07 (3 H, s), 2.09–2.20 (2 H, m), 2.45–2.53 (1 H, m), 2.64 (2 H, t, *J* 7.8), 3.01–3.06 (2 H, m), 3.98 (2 H, dt, *J* 2.5, 7.2), 4.87–5.00 (1 H, m), 6.94, 7.20 (4 H, AA′BB′, *J* 8.3), 7.31 (1 H, dd, *J* 1.6, 8.4), 7.39–7.48 (2 H, m), 7.62 (1 H, s), 7.73–7.81 (3 H, m); δ_C(75 MHz, $CDCl_3$) 14.2, 21.0, 22.7, 26.8, 27.6 (d, *J* 6.1), 31.1, 31.7, 34.2, 35.6, 36.4 (t, *J* 23.5), 37.8 (d, *J* 9.2), 51.1, 71.3 (t, *J* 20.1), 120.4 (t, *J* 246.3), 125.6, 126.2, 127.3, 127.5, 127.6, 127.8, 128.2, 130.0, 132.4, 133.7, 136.3, 139.6, 143.6, 170.1, 172.9 (one signal in the aromatic region not visible due to overlap); δ_F(282 MHz, $CDCl_3$) (–116.9) - (–115.8) (1F, m), –103.85 (1 F, d, *J* 239.0); *m/z* (MALDI-HRMS) 522.2818 (67%, $[M+H]^+$. $C_{32}H_{38}F_2NO_3^+$ requires 522.2814), 544.2628 (100, $[M+Na]^+$. $C_{32}H_{37}F_2NO_3Na^+$ requires 544.2634), 560.2375 (30, $[M+K]^+$. $C_{32}H_{37}F_2NO_3K^+$ requires 560.2373).

(1*SR*,4*RS*)-2,2-Difluoro-4-{[2-(2-naphthyl)ethyl](4-pentylphenyl)carbamoyl}cyclohexyl acetate ((±)-24). Using general procedure C, ketone (±)-**10** (1.000 g, 5.49 mmol) and DAST (1.450 cm^3, 1770 g, 10.98 mmol) in CH_2Cl_2 (30 cm^3) for 22 h, followed by CC purification (SiO_2; EtOAc/pentane 2:95), afforded olefin (±)-**13**.

Using general procedure F, olefin (±)-**13** (557 mg, 2.73 mmol), $RuCl_3$ (6.1 mg, 0.03 mmol), and $NaIO_4$ (2.334 g, 10.91 mmol) in MeCN/H_2O/CCl_4 (2:3:2) (18 cm^3) for 20 h yielded the crude carboxylic acid.

Using general procedure D, the crude acid and $SOCl_2$ (829 mm^3, 1.357 g, 4.28 mmol) in CH_2Cl_2 (1 cm^3) for 5 h yielded the crude acyl chloride (±)-**15**.

Using general procedure E, crude acyl chloride (±)-**15** (200 mg, 0.83 mmol), amine **22** (238 mg, 0.75 mmol), and DIPEA (145 mm^3, 107 mg, 0.83 mmol) in CH_2Cl_2 (5 cm^3) for 90 min, followed by CC purification (SiO_2; EtOAc/pentane 1:5), afforded (±)-**24** (124 mg, 18%) as a colourless oil. Found: C, 73.91; H, 7.40; N, 2.66. $C_{32}H_{37}F_2NO_3$ requires C, 73.68; H, 7.15; N, 2.66%; ν_{max}(solid)/cm^{-1} 3054, 2929, 2857, 1755 (CO), 1740, 1656 (CO), 1510, 1386, 1370, 1230, 1124, 1035, 973, 853; δ_H(300 MHz; $CDCl_3$) 0.93 (3 H, t, *J* 6.9), 1.33–1.53 (5 H, m), 1.55–1.59 (1 H, m), 1.61–1.71 (4 H, m), 1.75–1.91 (1 H, m), 1.86–2.03 (2 H, m), 2.05 (3 H, s), 2.65 (2 H, t, *J* 7.8), 3.60 (2 H, s), 3.64–3.76 (2 H, m), 5.07 (1 H, s), 6.96, 7.19 (4 H, AA′BB′, *J* 8.4), 7.17–7.20 (1 H, m), 7.38–7.46 (3 H, m), 7.67–7.80 (3 H, m); δ_C(75 MHz, $CDCl_3$) 14.0, 20.9, 22.7 (d, *J* 35.2), 26.8 (d, *J* 5.2), 29.6, 31.0, 31.4, 32.7 (d, *J* 8.9), 33.9 (t, *J* 22.6), 35.4, 41.5, 53.6, 68.7 (dd, *J* 22.6, 38.7), 120.7 (t, *J* 244.8), 125.4, 125.8, 127.2, 127.5, 127.5, 127.8, 128.1, 129.3, 129.6, 132.2, 132.7, 133.3, 139.6, 143.3, 169.4, 171.4; δ_F(282 MHz, $CDCl_3$) –105.72 (1 F, dd, *J* 29.8, 250.4), –102.58 (1 F, d, *J* 248.7); *m/z* (MALDI-HRMS) 522.2812 (81%, $[M+H]^+$. $C_{32}H_{38}F_2NO_3^+$ requires 522.2814), 544.2624 (100, $[M+Na]^+$. $C_{32}H_{37}F_2NO_3Na^+$ requires 544.2634), 560.2370 (30, $[M+Na]^+$. $C_{32}H_{37}F_2NO_3Na^+$ requires 560.2373).

(1*RS*,4*RS*)-3,3-Difluoro-4-hydroxy-*N*-[2-(2-naphthyl)ethyl]-*N*-(4-pentylphenyl)cyclohexanecarboxamide ((±)-25). Using general procedure G, acetate (±)-**23** (142 mg, 0.27 mmol) and an aqueous K_2CO_3/MeOH solution (1.0 g K_2CO_3 dissolved in 30 cm^3 H_2O and 115 cm^3 MeOH) (3.0 cm^3) in CH_2Cl_2 (0.5 cm^3) for 3 h, followed by CC purification (SiO_2; EtOAc/pentane 1:2), afforded (±)-**25** (113 mg, 87%) as a colourless oil. ν_{max}(solid)/cm^{-1} 2928, 2855, 2360, 2342, 1637 (CO), 1509, 1174, 1089, 948, 854, 668; δ_H(300 MHz; $CDCl_3$) 0.92 (3 H, t, *J* 6.9), 1.21–1.30 (1 H, m), 1.31–1.43 (4 H, m), 1.56–1.68 (4 H, m), 1.90–2.03 (1 H, m), 2.06–2.17 (1 H, m), 2.25 (1 H, d, *J* 5.9), 2.41–2.50 (1 H, m), 2.64 (2 H, t, *J* 7.8), 3.03 (2 H, dd, *J* 3.1, 6.2), 3.65–3.78 (1 H, m), 3.92–4.00 (2 H, m), 6.94, 7.20 (4 H, AA′BB′, $J = 8.1$), 7.31 (1 H, dd, $J = 1.9, 8.4$), 7.39–7.48 (2 H, m), 7.61 (1 H, s), 7.73–7.81 (3 H, m); δ_C(75 MHz, $CDCl_3$) 14.2, 22.6, 26.9, 30.1 (d, *J* 6.7), 31.0, 31.6, 34.1, 35.5 (d, *J* 9.2), 35.9 (d, *J* 23.8), 37.9 (d, *J* 9.8), 51.0, 70.9 (t, *J* 21.4), 121.6 (t, *J* 243.8), 125.3, 125.9, 127.1, 127.3, 127.3, 127.5, 127.5, 127.9, 129.8, 132.1, 133.4, 136.0, 139.3, 143.3, 172.9; δ_F(282 MHz, $CDCl_3$) (–121.2) - (–120.1) (1 F, m), –105.01 (1 F, d, *J* 233.7); *m/z* (MALDI-HRMS) 480.2715 (100%, $[M+H]^+$. $C_{32}H_{36}F_2NO_2^+$ requires 480.2709), 502.2541 (63, $[M+Na]^+$. $C_{32}H_{35}F_2NO_2Na^+$ requires 502.2528), 518.2285 (28, $[M+K]^+$. $C_{32}H_{35}F_2NO_2K^+$ requires 518.2267).

(1*RS*,4*RS*)-3,3-Difluoro-4-hydroxy-*N*-[2-(2-naphthyl)ethyl]-*N*-(4-pentylphenyl)cyclohexanecarboxamide ((±)-26). Using general procedure G, acetate (±)-**24** (120 mg, 0.23 mmol) and an aqueous K_2CO_3/MeOH solution (1.0 g K_2CO_3 dissolved in 30 cm^3 H_2O and 115 cm^3 MeOH) (3.0 cm^3) in CH_2Cl_2 (0.5 cm^3) for 3 h, followed by CC purification (SiO_2; EtOAc/pentane 1:2), afforded (±)-**26** (110 mg, quant.) as a colourless oil. Found: C, 75.06; H, 7.52; N, 2.92; F, 9.96. $C_{30}H_{35}F_2NO_2$ requires C, 75.13; H, 7.36; N, 2.92; F, 7.92%; ν_{max}(solid)/cm^{-1} 3364, 2928, 2856, 1634 (CO), 1510, 1420, 1317, 1167, 1141, 1090, 1062, 1025, 854, 818, 744; δ_H(300 MHz; $CDCl_3$) 0.91 (3 H, t, *J* 7.0), 1.24–1.35 (4 H, m), 1.35–1.43 (2 H, m), 1.59–1.69 (2 H, m), 1.83–1.95 (3 H, m), 2.37–2.44 (1 H, m), 2.41–2.52 (2 H, m), 2.64 (2 H, t, *J* 7.8), 3.01–3.07 (2 H, m), 3.80 (1 H, br s), 3.95–4.01 (2 H, m), 6.95, 7.19 (4 H, AA′BB′, *J* 8.4), 7.32 (1 H, dd, *J* 1.8, 8.4), 7.40–7.47 (2 H, m), 7.62 (1 H, s), 7.74–7.81 (3 H, m); δ_C(75 MHz, $CDCl_3$) 13.9, 21.6, 22.4, 28.2 (d, *J* 6.1), 30.8, 31.0, 31.5 (d, *J* 23.2), 34.0, 35.4, 37.8 (d, *J* 9.8), 51.0, 67.3 (m), 122.6 (t, *J* 242.6), 125.3, 125.9, 127.1, 127.3, 127.3, 127.5, 127.6, 127.9, 129.7, 132.1, 133.4, 136.1, 139.5, 143.1, 173.4; δ_F(282 MHz, $CDCl_3$) (–107.0) - (–106.0) (1 F, m), –102.80 (1 F, d, *J* 248.6); *m/z* (MALDI-HRMS) 480.2707 (100%, $[M+H]^+$. $C_{32}H_{36}F_2NO_2^+$ requires 480.2709), 502.2537 (43, $[M+Na]^+$. $C_{32}H_{35}F_2NO_2Na^+$ requires 502.2528), 518.2276 (8, $[M+K]^+$. $C_{32}H_{36}F_2NO_2K^+$ requires 518.2267).

(1*RS*)-3,3-Difluoro-4,4-dihydroxy-*N*-[2-(2-naphthyl)ethyl]-*N*-(4-pentylphenyl)-cyclohexanecarboxamide ((±)-27) and (1*RS*)-3,3-difluoro-*N*-[2-(2-naphthyl)ethyl]-4-oxo-*N*-(4-pentylphenyl)-cyclohexanecarboxamide ((±)-1). Using general procedure H, secondary alcohol (±)-**26** (592 mg, 1.23 mmol) and a 15% Dess–Martin periodinane solution in CH_2Cl_2 (3.836 cm^3, 5.225 g, 2.46 mmol) in CH_2Cl_2 (5.0 cm^3) for 7 h, followed by CC purification (SiO_2; EtOAc/pentane 1:3), afforded (±)-**1** (579 mg, 98%) as a colourless oil. If the crude product was not treated with molecular sieves, crystallization afforded fully hydrated (±)-**27**. (±)-**27**:

mp 83–87 °C; ν_{max}(solid)/cm^{-1} 3416, 2930, 2856, 1644 (CO), 1620, 1510, 1419, 1368, 1314, 1181, 1154, 1114, 1096, 1021, 974, 893, 822; δ_H(300 MHz; $CDCl_3$) 0.91 (3 H, t, *J* 6.9), 1.26–1.40 (5 H, m), 1.49–1.69 (5 H, m), 1.83–1.89 (1 H, m), 1.89–2.01 (1 H, m), 2.48–2.51 (1 H, m), 2.64 (2 H, t, *J* 7.8), 2.79 (1 H, s), 2.91 (1 H, s), 3.03 (2 H, dd, *J* = 5.8, 8.9), 3.95–4.00 (2 H, m), 6.94, 7.20 (4 H, AA′BB′, *J* 8.2), 7.31 (1 H, dd, *J* 1.6, 8.4), 7.40–7.47 (2 H, m), 7.61 (1 H, s), 7.73–7.81 (3 H, m); δ_C(75 MHz, $CDCl_3$) 14.2, 22.6, 25.3, 31.0, 31.6, 33.7 (d, *J* 22.6), 34.1, 34.6, 35.6, 37.8 (d, *J* 9.8), 51.1, 92.8 (dd, *J* 21.4, 28.0), 118.3 (t, *J* 244.9), 125.3, 125.9, 127.1, 127.3, 127.4, 127.5, 127.5, 127.9, 129.8, 132.1, 133.4, 136.0, 139.3, 143.3, 173.0; δ_F(282 MHz, $CDCl_3$) −120.9 (1 F, d, *J* 241.2), −111.4 (1 F, dd, *J* 29.9, 239.0); *m/z* (MALDI-HRMS) 478.2559 (100%, [*M*–OH]$^+$. $C_{30}H_{34}F_2NO_2^+$ requires 478.2552), 500.2388 (64, [*M* − OH_2+Na]$^+$. $C_{30}H_{34}F_2NO_2Na^+$ requires 500.2372), 516.2125 (30, [*M* − OH_2+K]$^+$. $C_{30}H_{34}F_2NO_2K^+$ requires 516.2111). (±)-**1**: ν_{max}(solid)/cm^{-1} 3376, 2928, 2855, 2360, 1756, 1634 (CO), 1511, 1418, 1365, 1323, 1275, 1183, 1108, 1080, 1027, 977, 819, 744; δ_H(400 MHz; $CDCl_3$) 0.84 (3 H, t, *J* 7.0), 1.25–1.30 (5 H, m), 1.54–1.62 (4 H, m), 1.87–1.93 (2 H, m), 2.15–2.46 (2 H, m), 2.58 (2 H, t, *J* 7.8), 2.97 (2 H, tt, *J* 3.8, 7.7), 3.90–3.95 (2 H, m), 6.90, 7.16 (4 H, AA′BB′, *J* 8.4), 7.23 (1 H, dd, *J* 1.7, 8.4), 7.35–7.38 (2 H, m), 7.54 (1 H, s), 7.65–7.73 (3 H, m); δ_C(75 MHz, $CDCl_3$) 14.2, 22.6, 29.1, 31.0, 31.6, 34.1, 35.6, 37.2 (d, *J* 9.2), 37.6, 38.6 (t, *J* 22.9), 51.1, 114.8 (dd, *J* 242.6, 258.5), 125.4, 126.0, 127.1, 127.2, 127.3, 127.5, 127.6, 128.0, 130.0, 132.1, 133.4, 135.8, 139.1, 143.6, 171.6, 196.7 (t, *J* 25.0); δ_F(282 MHz, $CDCl_3$) −114.13 (1 F, d, *J* 258.2), −104.0 - −102.9 (1 F, m); *m/z* (MALDI-HRMS) 478.2560 (100%, [*M*+H]$^+$. $C_{30}H_{34}F_2NO_2^+$ requires 478.2552), 500.2384 (49, [*M*+Na]$^+$. $C_{30}H_{33}F_2NO_2Na^+$ requires 500.2372), 516.2125 (20, [*M*+K]$^+$. $C_{30}H_{33}F_2NO_2K^+$ requires 516.2111).

X-Ray crystal structure of (±)-27

Crystal data at 233 K for $C_{30}H_{35}F_2NO_3$: M_r 495.61, orthorhombic, space group $Pn2_1a$ (non-standard setting of $Pna2_1$), D_x = 1.188 Mg m^{-3}, Z = 8, a = 15.0801(5) Å, b = 18.6531(7) Å, c = 19.7040(6) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 5542.6(3) Å^3. Bruker–Nonius KappaCCD diffractometer, MoKα radiation, λ = 0.71073, μ = 0.085 mm^{-1}. Crystal dimensions *ca.* 0.36 × 0.32 × 0.16 mm. The numbers of measured and independent reflections were 9715 and 9408, respectively. The structure was solved by direct methods (SIR-97).[44] All non H-atoms were refined anisotropically (disordered C-atoms, isotropically) by full-matrix least-squares analysis. The bond lengths of the disordered pentyl group were restrained to a value of 1.53 Å during refinement. H-positions are based on stereochemical considerations and were included in the structure factor calculation (SHELXL-97).[45] Final R(gt) = 0.0824, wR(gt) = 0.2026 for 635 parameters and 9408 reflections with $I > 2\sigma(I)$ and θ_{max} = 25.36°.†

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