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β-Lactams Derived from a Carbapenem Chiron Are Selective Inhibitors of Human Fatty Acid Amide Hydrolase versus Human Monoacylglycerol Lipase

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A library of 30 *β*-lactams has been prepared from (3*R*,4*R*)-3-[(*R*)-1′-(*t*butyldimethylsilyloxy)-ethyl]-4-acetoxy-2-azetidinone, and the corresponding deacetoxy derivative, by sequential *N*- and *O*-functionalizations with various *ω*-alkenoyl and *ω*-arylalkanoyl chains. All compounds were selective inhibitors of *h*FAAH versus *h*MGL, and IC_{50} values in the nanomolar range (5−14 nM) were recorded for the best representatives. From time-dependent preincubation and rapid dilution studies, and from docking analyses in a homology model of the target enzyme, a reversible mechanism of inhibition of *h*FAAH is proposed.

Introduction

The 2-azetidinone template (*β*-lactam) has been widely described as a lead structure for the inhibition of serine hydrolases such as human leukocyte elastase (HLE[a]),[1] prostate specific antigen (PSA),[2] thrombin,[3] human cytomegalovirus,[4] and mainly D,D-peptidases and *β*-lactamases, the bacterial target-enzymes of penicillin-type drugs used in antibiotherapy.[5] Generally, enzyme inhibition results from the interaction between the 2-azetidinone carbonyl and the active serine of the catalytic triad Ser-His-Asp. This interaction creates a covalent bond, via a tetrahedral intermediate, leading to a relatively stable acyl-enzyme complex, and therefore to the inhibition of the enzyme. Slow hydrolysis of the acyl-enzyme complex can regenerate the active enzyme, but in the case of so-called "suicide-substrates",[6] the inhibition is irreversible because the acyl-enzyme structure is no more sensitive toward hydrolysis. Surprisingly, the *β*-lactam motif has never been considered for fatty acid amide hydrolase (FAAH) inhibition, until our preliminary study which disclosed 3-alkenyl-2-azetidinones as micromolar inhibitors.[7] Like the above-mentioned enzymes, FAAH is a serine hydrolase but a member of a distinct class from the chymotrypsin family. Indeed, the active site differs from traditional enzymes by the replacement of Ser-His-Asp catalytic triad with Ser-Ser-Lys triad which constitutes the so-called amidase signature (AS).[8,9] Recently, a second AS enzyme has been discovered and termed FAAH-2;[10] regarding the original FAAH (also named FAAH-1), this enzyme shares only 20% sequence identity, but the same amide hydrolyzing activity using a Ser-Ser-Lys triad. FAAH exerts its activity on substrates possessing an amide bond, especially endogenous fatty acid amides (FAA). The principal substrate, and the most studied, is anandamide (arachidonoylethanolamide, AEA), a partial agonist of cannabinoid receptors CB_1 and CB_2.[11] Therefore, FAAH is commonly said to belong to the endocannabinoid system which consists of different hydrolases: FAAH-1, FAAH-2, monoacylglycerol lipase (MGL),[12] and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA),[13] among others.[14−17] MGL and NAAA preferentially hydrolyze 2-arachidonoylglycerol (2-AG) or 2-oleoylglycerol (2-OG) and palmitoylethanolamide, respectively. FAAH hydrolyses anandamide, other endogenous fatty acid amides, but also a particular class of *N*-acylamino acids, that is, *N*-acyl taurines (NATs) which activate transient receptor potential (TRP) ions channels,[18] and oleamide,[19] a fatty acid primary amide recognized as a sleep-inducing lipid. The actual knowledge of these bioactive lipids and the role played by FAAH in the control of their levels open the door to the development of novel therapeutic agents.[20] Indeed, pharmacological investigations in animal models have shown that a large number of biological benefic effects such as appetite stimulation, anti-inflammatory effects, sleep induction,[21] anxiety release and analgesia[22,23] could be enhanced by controlling FAAH catabolic activity.

The search of FAAH inhibitors constitutes a domain of growing interest which has been recently reviewed.[24,25] Potent inhibitors based on different types of electrophilic functions

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[a] Abbreviations: *h*FAAH, human fatty acid amide hydrolase; *h*MGL, human monoacylglycerol lipase; AS, amidase signature; FAAs, fatty acid amides; AEA, anandamide; CB_1, cannabinoid receptor subtype-1; CB_2, cannabinoid receptor subtype-2; NAAA, *N*-acylethanolamine hydrolyzing acid amidase; 2-AG, 2-arachidonoylglycerol; 2-OG, 2-oleoylglycerol; BTNP, (*E*)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one; DCM, dichloromethane; ACN, acetonitrile; DCC, dicyclohexylcarbodiimide; DMAP, dimethylaminopyridine; HMDS, hexamethyldisilazane; CBz, benzyloxycarbonyl; TBDMS, *tert*-butyldimethylsilyl; MAFP, methyl arachidonyl fluorophosphonate; rmsd, root-mean-square deviation; ACB, acyl chain binding; CA, cytoplasmic access; DMSO, dimethyl sulfoxide.

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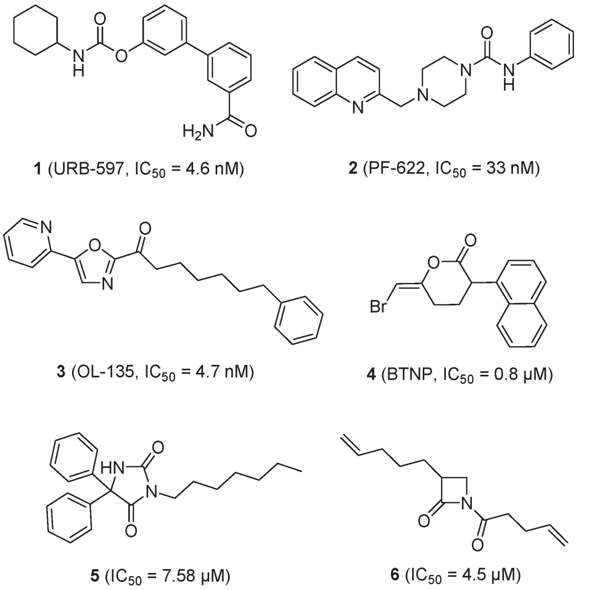


1 (URB-597, IC_{50} = 4.6 nM) **2** (PF-622, IC_{50} = 33 nM)

3 (OL-135, IC_{50} = 4.7 nM) **4** (BTNP, IC_{50} = 0.8 μM)

5 (IC_{50} = 7.58 μM) **6** (IC_{50} = 4.5 μM)

Figure 1. Structures of previously described FAAH inhibitors (the mentioned IC_{50} is the one reported by the respective authors, against rat enzyme).

have been published. They are divided into two mechanistic classes: irreversible carbamates[26−31] and ureas[32,33] inhibitors, which include the pharmacological tools **1** (URB-597)[34] and **2** (PF-622),[33] and the reversible α-keto oxazoles[35−39] inhibitors (and other heterocycles) illustrated by **3** (OL-135)[23] (Figure 1). Reaction of **1** and **2** with FAAH leads to inactive and stable acyl-enzymes. Initial proton exchange between Lys142, Ser217, and Ser241 (catalytic triad) allows the nucleophilic attack of Ser241 on the carbonyl function of the inhibitor; the resulting tetrahedral intermediate expulses the leaving group, namely, the phenol moiety of **1** or the aniline group of **2**, along with proton transfer from Ser217, thus leading to Ser241 covalently modified as a carbamate. The postulated mechanism of FAAH interaction with **3** starts similarly, but since the tetrahedral intermediate features no leaving group, reversible inhibition occurs. Within this family of covalent reversible inhibitors, SAR studies have clearly shown that the activity is linked to the electrophilic character of the ketone.[35]

Embedding the sensitive carbonyl function into a cyclic structure appears to be a quite unusual strategy for the design of FAAH inhibitors. (*E*)-6-(Bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one (**4**, Figure 1) was an early covalent inhibitor of anandamide hydrolysis.[40] A unique series of (thio)hydantoin-based FAAH inhibitors, exemplified with 3-heptyl-5,5′-diphenylimidazolidine-2,4-dione (**5**, Figure 1), has been reported by Muccioli et al.[41] Such molecules act as competitive inhibitors without being hydrolyzed by the enzyme. Lastly, a few lipophilic *β*-lactams were shown to be modest inhibitors of FAAH: 3-(4′-pentenyl)-1-(4′-pentenoyl)-2-azetidinone (**6**, Figure 1) emerged as a micromolar inhibitor.[7] Starting from this preliminary result, we have investigated the synthesis and the pharmacological properties of a new family of FAAH inhibitors, derived from acetoxy-azetidinone **7**, in order to possibly improve the activity. The structures were decorated with different acyl chains on N1 and C5−O positions, featuring a terminal phenyl (Ph), biphenyl (biPh), or alkene (Alk) motif as found on the hydrophobic scaffolds of traditional FAAH inhibitors. A series of 30 azetidinones was evaluated in vitro for the inhibition of human FAAH (*h*FAAH) and human MGL (*h*MGL). The most promising compounds were submitted to a docking study in a new model of *h*FAAH.

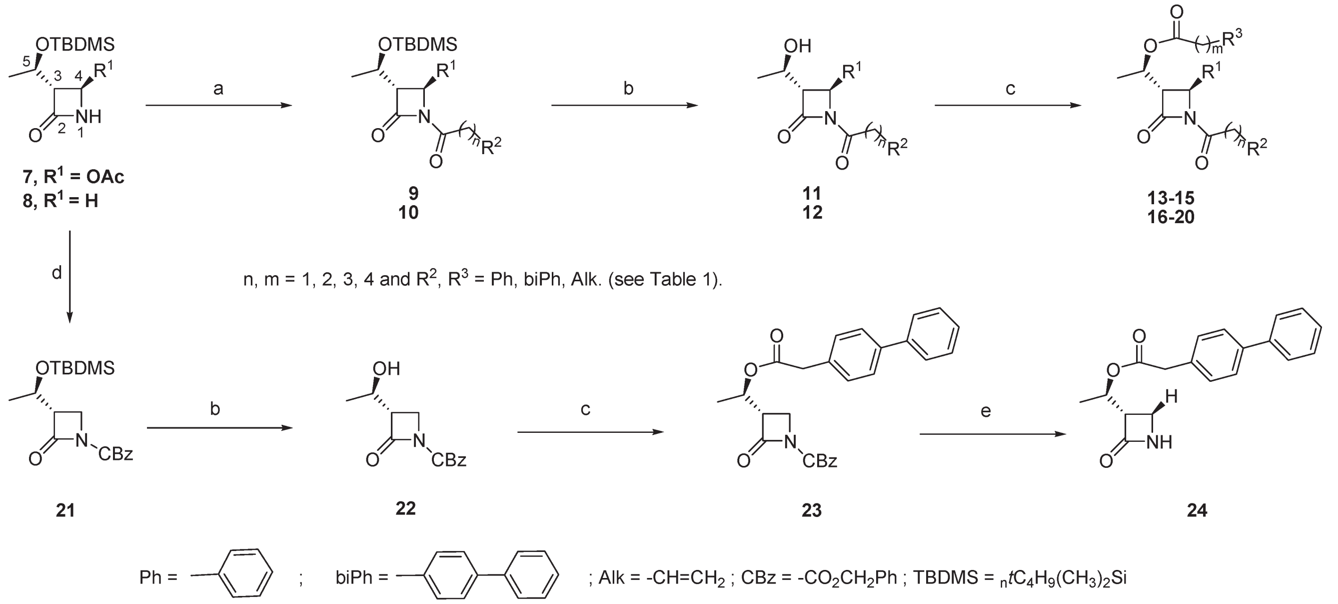
Results and Discussion

Synthesis. Acetoxy-azetidinone **7** is a commercially available chiral precursor of (carba)penems antibiotics.[42] This molecule offers several advantages: (i) the amide function can be easily substituted on the N1 position; (ii) after deprotection of the silyl ether group, the hydroxyl function of the side-chain can also be substituted (C5−O position); (iii) the acetoxy substituent (OAc) on the C4 position increases the heterocycle chemical reactivity (N1−C2 cleavage) by its electron withdrawing effect; (iv) OAc is also a good leaving group. This last structural feature would make possible the occurrence of an irreversible suicide-type inhibition, if a serine hydrolase enzyme reacted on the *β*-lactam ring. Moreover, the chemical reactivity of the OAc substituent allows its formal elimination by a two-step sequence of reactions (substitution/reduction), giving the less hindered and more stable precursor **8** (Scheme 1).

A first family of lipophilic azetidinones was prepared from **7**, taking inspiration from previously described protocols (Table 1, entries 1−10).[43,44] Briefly, **7** was *N*-acylated by reaction with hydrocynnamoyl chloride, 4-phenyl-butanoyl chloride, or 4-pentenoyl chloride, and pyridine, in refluxing dichloromethane (DCM), to furnish respectively azetidinones **9a** (89%), **9b** (94%), and **9c** (80%). The silyl ether function was deprotected by treatment with HCl-HOAc at −5 °C. The resulting alcohols **11a−c** (83−99%) were directly engaged in esterification reactions with hydrocynnamoyl chloride, 4-phenyl-butanoyl chloride, or 4-pentenoyl chloride, in the presence of pyridine at room temperature, giving the following bis-acylated compounds: **13a** (99%), **14a** (88%), **13b** (70%), **14b** (52%), and **15e** (90%). The biphenylacetyl side chain was introduced by an alternative method: the reaction of **11a,b** with biphenylacetic acid and dicyclohexylcarbodiimide (DCC), in the presence of dimethylaminopyridine (DMAP) as catalyst. Compounds **13d** (67%) and **14d** (77%) were isolated.

A second family of compounds (Table 1, entries 11 to 31) was prepared from **8**.[45] This precursor could be readily obtained by substitution of **7** with thiophenolate followed by reduction with tris(trimethylsilyl)silane hydride (see Supporting Information). As above, **8** reacted with hydrocynnamoyl chloride, 4-phenyl-butanoyl chloride, 5-phenylpentanoyl chloride, 4-pentenoyl chloride, or 5-hexenoyl chloride to afford respectively the *N*-acylated azetidinones **10a** (88%), **10b** (87%), **10c** (74%), **10d** (95%), and **10e** (46%) (see Scheme 1). After *t*-butyldimethylsilyl deprotection under acidic conditions, the resulting alcohols **12a−e** (78−94%) were esterified with various acid chlorides and pyridine (Method A), or with the corresponding carboxylic acids, DCC and DMAP (Method B). Application of the Method A to hydrocynnamoyl chloride and **12a,b** gave the azetidinones **16a** (79%) and **17a** (89%). From 4-phenylbutanoyl chloride and **12a−e** were obtained respectively **16b** (75%), **17b** (87%), **18b** (63%), **19b** (84%), and **20b** (77%). Reaction of 4-pentenoyl chloride with **12d** furnished 19e (88%). Applying the Method B to **12a,b** and 5-phenylvaleric acid, we produced the bis-acylated azetidinones **16c** (59%) and **17c** (93%). Similarly, from biphenylacetic acid

Scheme 1. Synthesis of Substituted Azetidinones[a]



[a] Reagents and conditions: (a) acyl chloride, pyridine, DCM, 45 °C, 24 h; (b) HCl, AcOH, ACN, −5 °C to rt, 3 h; (c) acyl chloride, pyridine, DCM, rt, 15 h or carboxylic acid, DCC, DMAP, DCM, rt, 15 h; (d) benzyl chloroformate, LiHMDS, −78 °C to rt, 4 h; (e) H_2, Pd/C, EtOH/AcOEt, 1 h.

Table 1. Determination of the Inhibitory Potential of Azetidinones Towards Human FAAH and Human MGL[a]

entry	compound	R^1	*n*	R^2	*m*	R^3	IC_{50} *h*FAAH[b]	% inhibition (MGL)[c]	IC_{50} *h*MGL[b]
1	**11a**	OAc	2	Ph			223.6	48	
2	**11b**	OAc	3	Ph			182.8	61	
3	**11c**	OAc	2	Alk			537.0	16	
4	**13a**	OAc	2	Ph	2	Ph	2.02	100 (0)	
5	**13b**	OAc	2	Ph	3	Ph	0.96	100 (0)	
6	**13d**	OAc	2	Ph	1	biPh	0.826	66	
7	**14a**	OAc	3	Ph	2	Ph	5.12	100 (0)	
8	**14b**	OAc	3	Ph	3	Ph	3.12	100 (0)	
9	**14d**	OAc	3	Ph	1	biPh	0.708	100 (0)	
10	**15e**	OAc	2	Alk	2	Alk	1.9	99 (33)	133
11	**12a**	H	2	Ph			408.7	8	
12	**12b**	H	3	Ph			nd	nd	
13	**12c**	H	4	Ph			nd	nd	
14	**12d**	H	2	Alk			7.9	89 (8)	
15	**12e**	H	3	Alk			nd	nd	
16	**16a**	H	2	Ph	2	Ph	0.157	100 (0)	
17	**16b**	H	2	Ph	3	Ph	0.049	100 (0)	
18	**16c**	H	2	Ph	4	Ph	0.091	100 (0)	
19	**16d**	H	2	Ph	1	biPh	0.050	31	
20	**17a**	H	3	Ph	2	Ph	0.057	54	
21	**17b**	H	3	Ph	3	Ph	0.030	100 (0)	
22	**17c**	H	3	Ph	4	Ph	0.045	59	
23	**17d**	H	3	Ph	1	biPh	0.032	0	
24	**18b**	H	4	Ph	3	Ph	0.449	39	
25	**18d**	H	4	Ph	1	biPh	0.236	25	
26	**19b**	H	2	Alk	3	Ph	0.005	89	4.06
27	**19d**	H	2	Alk	1	biPh	0.012	91	1.84
28	**19e**	H	2	Alk	2	Alk	0.098	99	23.3
29	**19f**	H	2	Alk	3	Alk	0.032	8	4.72
30	**20b**	H	3	Alk	3	Ph	0.010	85	8.51
31	**20d**	H	3	Alk	1	biPh	0.014	67	14.6
32	**24**	H			1	biPh	6.5	16	

[a] See Supporting Information for the corresponding table of pI_{50} values and Standard Error. [b] IC_{50} in μM (from three independent experiments) [c] Percentage of inhibition at 10^{-4} M. The percentage of inhibition at 10^{-6} M is stated between brackets.

and **12a−e**, we prepared the compounds **16d** (93%), **17d** (83%), **18d** (81%), **19d** (68%), and **20d** (66%). Lastly, reaction of **12d** with 5-hexenoic acid gave the azetidinone 19f (84%).

For comparison purposes (see below, enzymatic tests), one representative azetidinone monosubstituted at the C5−O position was prepared in four steps (Scheme 1 and Table 1, entry 32). Amide protection of **8** with a benzyloxycarbonyl group (**21**, 99%), silyl ether deprotection as usual (**22**, 91%), esterification with biphenylacetic acid (**23**, 83%), and N1 deprotection by catalytic hydrogenation afforded the azetidinone **24** (96%; overall yield for four steps, 72%).

All final azetidinones and intermediates were fully characterized by the usual spectroscopies (see Experimental Section). Typical features are exemplified with **14d** (first series, R^1 = OAc) and **19b** (second series, R^1 = H). ^{1}H NMR spectrum of **14d** shows the vicinal β-lactamic protons with the *trans* relationship at 3.28 ppm (H3, dd, J = 6.5 and 1.7 Hz) and 6.46 ppm (H4, d, J = 1.7 Hz); four carbonyl signals are visible in ^{13}C NMR at 170.4 (O−CO), 169.8 (N-CO), 169.1 (OAc) and 162.2 (β-lactam carbonyl) ppm; the IR spectrum shows the carbonyl stretchings at 1803 (β-lactam), 1740 (broad, OAc and ester), and 1717 (imide) cm^{-1}. For **19b**, the geminal β-lactamic protons H4/H4′ appear in ^{1}H NMR as a typical ABX pattern at 3.53 ppm (dd, J = 7.7 and 3.7 Hz) and 3.66 ppm (dd, J = 7.7 and 6.6 Hz), while H3 gives a multiplet at 3.40 ppm; the ^{13}C NMR spectrum shows three carbonyl signals at 172.4 (O−CO), 170.3 (N-CO), and 164.4 (β-lactam CO) ppm, and the IR spectrum shows the carbonyl stretchings at 1786 (β-lactam), 1734 (ester), and 1703 (imide) cm^{-1}. In both series (R^1 = OAc or H), H5 proton of precursors **9,10** (silyl ether) and **11,12** (free alcohol) gives a multiplet (qd) around 4.3 δ in ^{1}H NMR spectra; after the *O*-acylation leading to the final compounds **13−15** and **16−20**, a deshielding of about 1 δ is observed (H5 around 5.3 δ). The chemical and enantiomeric purity of all tested compounds has been controlled by HPLC, using C18 and AD-H columns, respectively.

Biochemical Evaluation

The azetidinones listed in Table 1 have been tested as potential inhibitors of *h*FAAH and *h*MGL. Human recombinant enzymes, developed in our laboratory,[46,47] were used in competitive hydrolytic assays using [^{3}H]-radiolabeled AEA and [^{3}H]-radiolabeled 2-OG, respectively, as substrates. Tested compounds, enzymes, and [^{3}H]-substrates were incubated at 37 °C during 10 min. The inhibition rates were evaluated by liquid scintillation counting (LSC) of the restitual hydrolysis products of the labeled substrates. The results reported in Table 1 are the means of three independent assays.

FAAH Inhibition. Collected results clearly show that the azetidinones equipped with only one acyl chain, at N1 position (entries 1−3 and 11−15) or C5−O position (entry 32), are modest or very weak inhibitors of FAAH. Among the compounds bearing two acyl chains, fixed at N1 and C5−O positions, the first series (R^1 = OAc, entries 4−10) systematically appears less active than the second one (R^1 = H, entries 16−31). Our initial hypothesis that the C4 acetate substituent would improve the azetidinone inhibitory effect − by increasing the chemical reactivity of the heterocycle (electronwithdrawing effect) and/or by initiating an enzymatic suicide-mechanism (leaving group effect) − turned out to be contradicted by these first results. Accordingly, the discussion focuses only on the second series of disubstituted azetidinone inhibitors **16a−d**, **17a−d**, **18b,d**, **19b−f**, and **20b,d** which are potent FAAH inhibitors. The studied factors were the chain length (n, m = 1−4) and the nature of the end group (Ph, biPh, Alk) for both substituted positions (N1, C5−O). All compounds **16−20** revealed to be good inhibitors of *h*FAAH with IC$_{50}$ values ranging from 0.005 μM (**19b**) to 0.45 μM (**18b**). Comparatively to our previous “hit” (structure **6**, Figure 1; IC$_{50}$ = 4.5 μM), the activities have been significantly improved. On the basis of the results reported here, some structure−activity relationships can be drawn. Compounds **16**, with *N*-(3-phenylpropanoyl) chain (entries 16−19), and **12**, with *N*-(5-phenyl-pentanoyl) chain (entries 24 and 25), are less potent than their corresponding analogues **17**, with *N*-(4-phenylbutanoyl) chain (entries 21−23). Compounds **19**, with *N*-(4-pentenoyl) chain (entries 26−29), are slightly more potent than their corresponding analogues **20**, with *N*-(5-hexenoyl) chain. Within subfamilies, compounds named **b**, with *O*-(4-phenyl-butanoyl) chain (entries 17, 21, 26, 30), and d, with *O*-(biphenyl-acetyl) chain (entries 19, 23, 27, 31), are the best inhibitors. We concluded that similar activities result from the presence of 4-phenyl-butanoyl (n = 3, R^2 = Ph) and 4-pentenoyl (n = 2, R^2 = Alk) substituents at the N1 position, on the one hand, and from the presence of 4-phenyl-butanoyl (m = 3, R^3 = Ph) and biphenylacetyl (m = 1, R^3 = biPh) substituents at the C5−O position, on the other hand.

MGL Inhibition. Azetidinones **11**, **13−14** of the first series (R^1 = OAc) inhibited the enzyme at 10^{-4} M concentration (50−100% inhibition), but not at 10^{-6} M concentration (Table 1, entries 1 to 10). An IC$_{50}$ value of 133 μM was determined for the most active compound **15e** (entry 10) which, however, shows a great selectivity for the inhibition of FAAH (IC$_{50}$ = 1.90 μM).

Azetidinones **12**, **16−20** of the second series (R^1 = H) were also modest inhibitors of MGL (entries 11−25). IC$_{50}$ values of the most active azetidinones **19b−f** and **20b,d** ranged from 1.84 to 23.3 μM (entries 26−31). Here again, the selectivity versus FAAH inhibition is high: for instance, **19b** (entry 26) and **20b** (entry 30) are respectively 800 and 850 times more potent against FAAH. For the other compounds, **19d−f** and **20d**, the selectivities range within 100 and 240.

Inhibition Mode. To determine the likely mechanism of FAAH inhibition, two types of experiments were performed, that is, time-dependent preincubation and rapid dilution studies, both using azetidinones **19b** (IC$_{50}$ = 0.005 μM) and **19f** (IC$_{50}$ = 0.032 μM). Concerning the preincubation study, it is expected with an irreversible-type inhibitor that the inhibitor potency should increase upon prolonged preincubation time. Conversely, a constant IC$_{50}$ value upon preincubation supports a reversible mechanism of inhibition.[48] Thus, **19b** and **19f** were incubated with the enzyme for 0, 15, 45, or 90 min, prior to substrate addition. As illustrated in Figure 2, the preincubation had no effect on the inhibiting activity of the compounds. This suggests an inhibition mode similar to those of α-keto-oxazoles (see **3**, Figure 1) or hydantoins (see **5**, Figure 1).[35,41] On the other hand, after rapid and large dilution of the inhibitor−enzyme mixture, the recovery of enzymatic activity should be almost total if the inhibitor is reversible. For the irreversible inhibitors, the enzyme remains largely inhibited because the inhibitor is bound to the enzyme. Here, the rapid and large dilution led to a recovery of enzymatic activity in the case of **19b** and **19f**, as for 1-oxazolo[4,5-b]pyridin-2-yl-6-phenyl-1-hexanone (CAY10402),[49] an analogue of **3** (Figure 1). As a further control we used two irreversible FAAH inhibitors, compound **1** (Figure 1) and methyl arachidonyl fluorophosphonate (MAFP)[50] and found that the enzyme activity was still profoundly inhibited after the dilution (Figure 3a).

Furthermore, the mechanism of **19b** interaction with *h*FAAH was determined by studying the velocity of anandamide

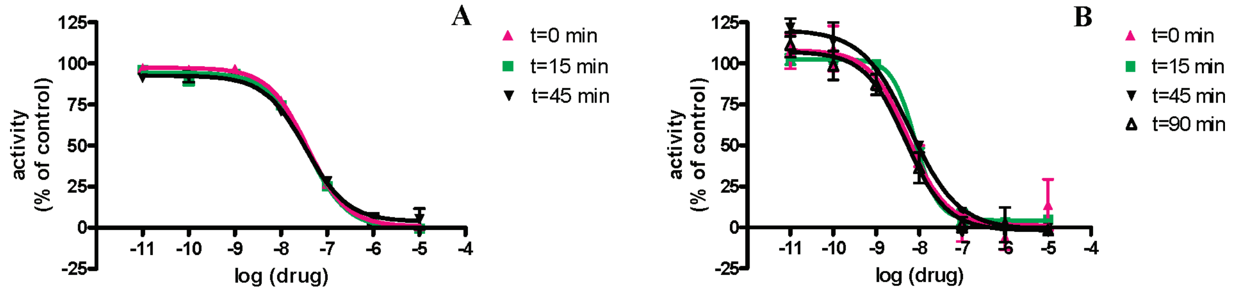


Figure 2. Determination of the mode of inhibition of **19f** (A) and **19b** (B). The influence of the time of preincubation (0, 15, 45, and 90 min) on the inhibition curves of *h*FAAH was studied resulting in no significant variation of the IC_{50} values.

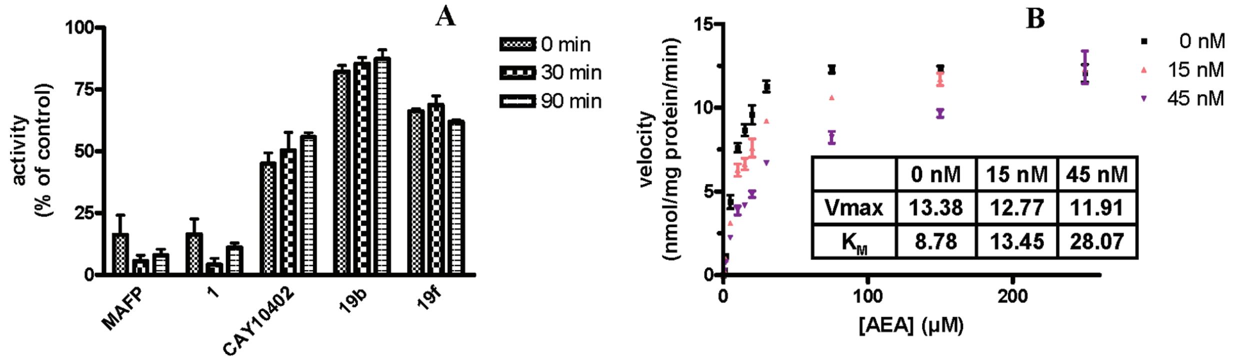


Figure 3. (A) Test of reversibility: influence of a rapid and large dilution on the recovery of *h*FAAH activity (studies after 0, 30, and 90 min following the rapid and large dilution). (B) Determination of the mechanism of **19b** interactions with *h*FAAH. Michaelis–Menten curves and rapid dilution graphs were obtained from three independent experiments. The kinetic parameters are shown in the inset (V_{max} values are given as nmol min^{-1} mg^{-1} of protein and K_M values are in μM).

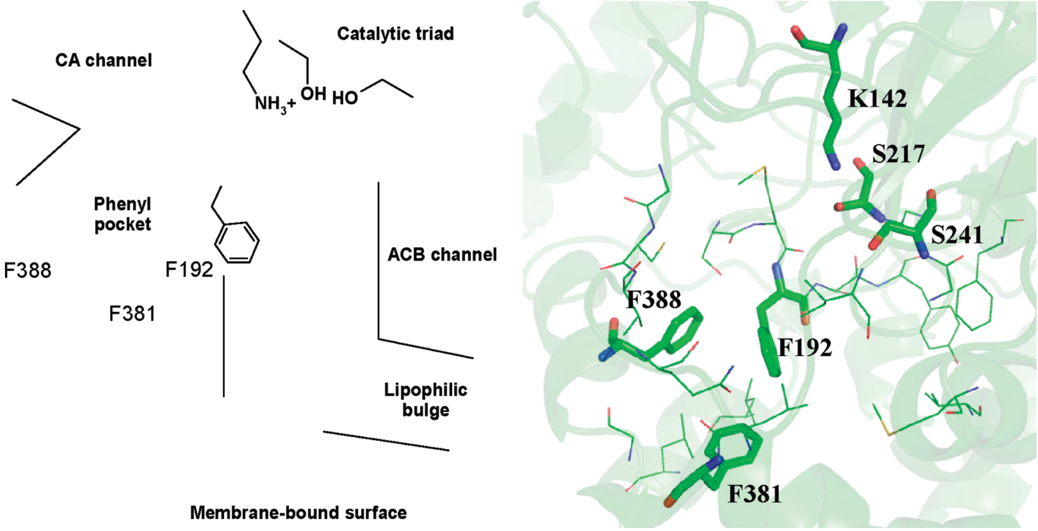


Figure 4. Representation of the active site of the modeled human FAAH.

metabolism in function of increasing concentration of anandamide. The Michaelis–Menten curves (Figure 3b) and resulting kinetic parameters suggest a competitive inhibition type for this compound. Indeed, the V_{max} values in the presence of 15 or 45 nM of **19b** (12.77 ± 0.22 and 11.91 ± 0.50 nmol min^{-1} mg^{-1}, respectively) are similar to the V_{max} value obtained in the absence of inhibitor (13.38 ± 0.25 nmol min^{-1} mg^{-1}), whereas the K_M values are largely increased in the presence of inhibitor.

On the basis of these data, to collect more information about the possible enzyme–inhibitor interactions at the atomic level, a modeling study has been performed.

Thereotical Study

Model of the Human FAAH. The crystal structure of *h*FAAH is currently not available. But recently, an engineered form of rat FAAH showing the same activity profile as the human one was crystallized (PDB code 2VYA).[51] We therefore decided to build a model of *h*FAAH through homology modeling using this X-ray crystal structure. Their amino acid sequence shared 80.6% identity. The EsyPred3D program was used.[52] This automated homology modeling tool compares results from various multiple alignment

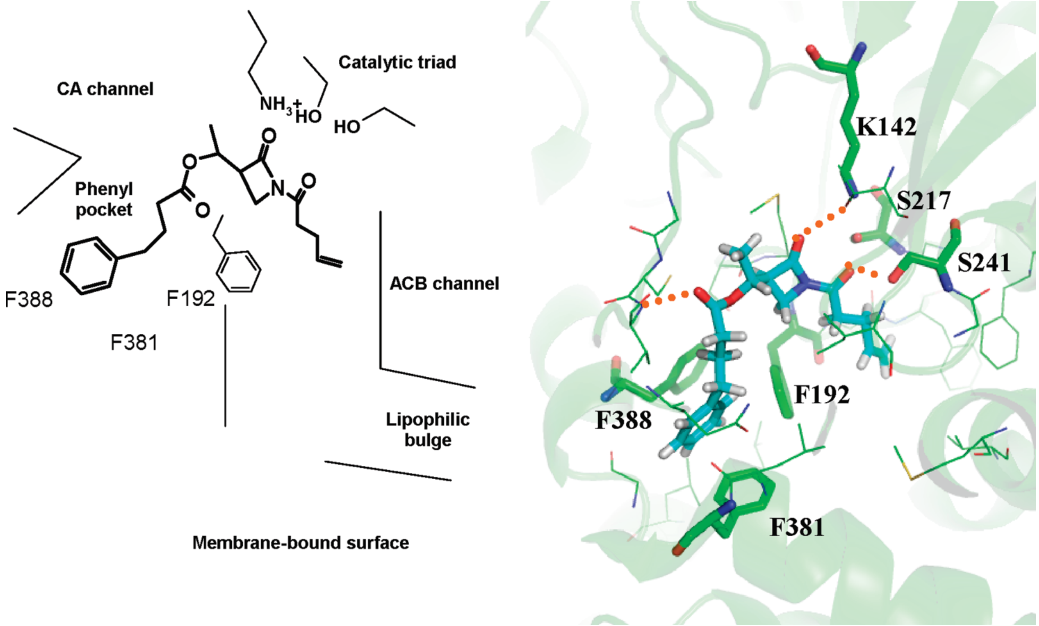


Figure 5. Proposed binding mode **I** of **19b** into the human FAAH. In the panel on the right, H bonds are depicted by orange dotted lines.

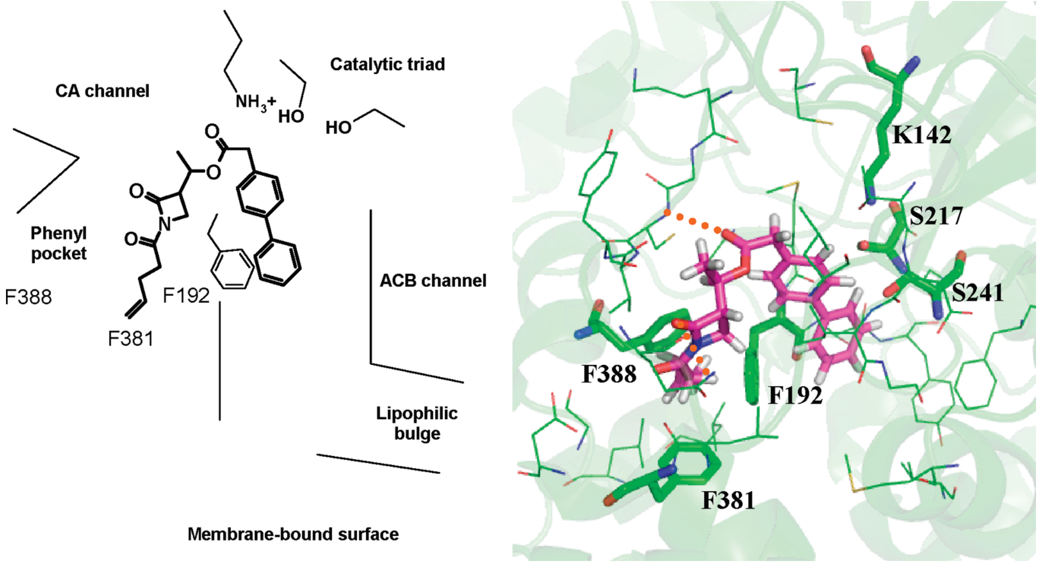


Figure 6. Proposed binding mode **II** of **19d** into the human FAAH. In the panel on the right, H bonds are depicted by orange dotted lines.

algorithms to derive a "consensus" alignment between the target sequence and the template. Quality verification of the model was performed with Procheck 3.0 with a pseudoresolution of 2.8 Å.[53] The model obtained is reliable based on the Ramachandran plot, showing 91.2% of the residues in the core regions and 8.6% in the allowed one (see Supporting Information). Moreover, 99.1, 94.8, and 100.0% of the main chain bond lengths, main chain bond angles, and the planar groups, respectively, are within the standard geometries. The root mean square deviation (rmsd) for the backbone atoms between both structures is 0.091 Å. The active site of *h*FAAH is formed by a hydrophobic tunnel, called the acyl chain binding channel (ACB), leading from the membrane-bound surface to the hydrophilic catalytic triad (Ser241, Ser217, and Lys142) (Figure 4). From the membrane, the ACB channel bifurcates into a lipophilic bulge. A second tunnel, the cytoplasmic access channel (CA), is exposed to the solvent and emerges at about an 80° angle from the ACB channel. A third channel composed of three phenylalanine residues (Phe388, Phe381, and Phe192), here called the "phenyl pocket", lies close to the ACB channel.

Docking Studies. Since the above-described pharmacological data suggest that these compounds are competitive inhibitors we docked the most active inhibitors, **19b** and **19d**, into the substrate binding site of the modeled *h*FAAH to further understand their binding mode. Therefore we used the GOLD program, which we used for a previous work on FAAH,[54] to dock these compounds into the active site of our human FAAH model. Recent theoretical and structural studies showed the planarity of the amide *β*-lactam bond and that the two imide carbonyls (called here COlactam and COexo) can adopt either *E* or *Z* configuration, the *E* configuration being the more stable in the gas phase (ΔE = 3.9 kcal/mol).[43] We therefore allowed flipping the imide

Table 2. Characteristics of the Two Proposed Binding Modes of Azetidinone Compounds Inside the Modelled Human FAAH

compound	binding mode	configuration of the imide carbonyls	H bonds	distance (Å)
19b	**I**	*Z*	COexo···OH(Ser241)	2.24
			COlactam···OH(Thr236)	3.26
			COester···NH(Val270)	2.95
19d	**II**	*Z*	COester···NH(Cys269)	3.32
	I	*E*	COlactam···OH(Ser241)	3.07

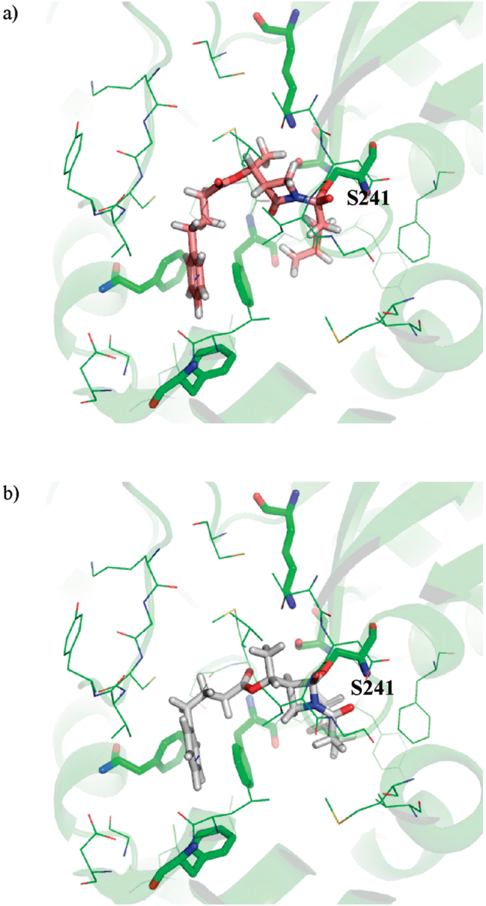


Figure 7. Binding mode of the putative tetrahedral intermediates of **19b**, binding Ser241 either (a) via the exocyclic imide carbonyl (COexo) or (b) via the lactam carbonyl (COlactam) in the modeled human FAAH.

bond during the docking run. On the basis of GOLD scoring function and on the occurrence of the docking poses, two preferential binding modes were retained (Figures 5 and 6; Table 2). We observed a *Z* or *E* configuration of the imide carbonyls following the binding mode and the studied compound. In the first binding mode (**I**) (Figure 5), the phenyl or biphenyl chain lies in the "phenyl pocket" and interacts with the three phenylalanines Phe192, Phe381, and Phe388. The catalytic serine Ser241 is close to the lactam and imide carbonyls. The alkene chain lies at the beginning of ACB channel and is close to Phe192. The observed H bonds are described in Table 2. In the second binding mode (**II**) (Figure 6), only observed for **19d**, the biphenyl and alkene chains are located in the ACB channel and "phenyl pocket", respectively. The biphenyl group interacts with Phe192.

In both binding modes, several amino acids of the active site are involved in hydrophobic contacts with the inhibitors (see Supporting Information). From our docking experiments, we can explain the optimal chain length $m = 3$ (phenyl) or $m = 1$ (biphenyl), and $n = 2$ (alkenyl), by the stabilizing $\pi-\pi$ interactions between the phenyl/biphenyl or alkene group and phenylalanine residues of the active site. Moreover, in both cases, mode **I** or **II**, adding an acetate moiety in the lactam cycle at C4 would lead to steric hindrance. The same binding modes were also observed for **16b** and **16d** (results not shown).

The first binding mode (Figure 5) could suggest a mechanism of action similar to that of α-keto heterocycles acting as reversible, competitive inhibitors presumably via reversible hemiketal formation with the active serine Ser241.[35,55] In this context, we did a covalent docking of the two putative tetrahedral intermediates of **19b**, binding Ser241 either via the lactam carbonyl (COlactam) or via the exocyclic imide carbonyl (COexo). In both cases, the position of the inhibitor is close to the one of the first binding mode with the phenyl group interacting with the "phenyl pocket" and the alkene chain lying in the ACB channel (Figure 7). The anion oxygen interacts by H bonding with the oxyanion hole, that is, with the backbone of Ile238, Gly239, and Gly240. For the intermediate via the exocyclic carbonyl, both *Z* and *E* configurations are observed with a highest occurrence for *E*.

Following the second binding mode (Figure 6), the inhibitors would rather act as the (thio)hydantoin inhibitors, described recently, without tetrahedral intermediate.[54]

As an internal validation of the docking methodology, the inhibitor *N*-phenyl-4-(quinolin-3-ylmethyl)piperidine-1-carboxamide (PF-750)[33] covalently attached to the Ser241 and used to generate the published crystal structure of the humanized form of rat FAAH,[51] was redocked into the empty catalytic pocket of the crystal structure using the same docking protocols. The conformation of the top scoring pose could reproduce the crystal structure conformation (data not shown), validating the docking methodology.

Conclusion

Till now, *β*-lactams were not considered as potential pharmacologically active compounds to interact with the endocannabinoid system in humans. In 2008, the virtual screening of a database of about 500 000 Shering-Plough compounds by using a CB_1 pharmacophore model as filter, and additional constraints for drug-like structures, allowed selection of 420 compounds for further in vitro evaluation. Among them, a series of five diaryl 2-azetidinones emerged, giving an inhibition rate of ≥50% at 100 nM in a CB_1 competitive binding assay. From this nonorientated approach, one *β*-lactam "lead" compound was identified as novel CB_1 receptor antagonist with a K_i value of 53 nM.[56]

To our knowledge, the design of potentially active *β*-lactams in the cannabinoid system was not reported before. Our approach was simply based on the FAAH inhibition by using the *β*-lactam core as electrophilic carbonyl function; this heterocycle was equipped with lateral chains mimicking the natural substrates or the known inhibitors, and susceptible to make hydrophobic contacts in the active site of the target enzyme.

Starting from the chiral 2-azetidinone **7** traditionally used for the synthesis of antibiotics, we generated a variety of lipophilic derivatives by placing alkenoyl, phenylalkanoyl, and biphenylacetyl chains on positions N1 and C5−O. Evaluation of this library of 30 azetidinones against *h*FAAH and *h*MGL revealed good to excellent and selective inhibitors of *h*FAAH versus *h*MGL, with IC_{50} values of 5−14 nM for the best representatives (**19b**, **19d**, **20b**, and **20d**). Since the IC_{50} values were constant upon prolonged incubation time and as total recovery of enzymatic activity was observed after rapid and large dilution, a reversible mechanism of inhibition can be proposed. In addition, as the V_{max} values are not affected by the presence of **19b** while the K_M values are increased, the interaction between **19b** and *h*FAAH is likely to be of a competitive type. This is a quite unexpected result, since the *β*-lactams are prone to form (more or less) stable acyl-enzyme intermediates with serine hydrolases. Note that docking studies of two potent inhibitors into a validated homology model of *h*FAAH support well the reversible mechanism, even though they do not allow discriminating between two binding modes, with either the lactam/imide carbonyls or the ester carbonyl facing the catalytic triad. Further studies are in progress to clarify the role played by each carbonyl function of the inhibitors **16−20** and to identify the carbonyl function possibly responsible for the formation of a reversible tetrahedral intermediate by reaction with the active serine.

Experimental Section

Chemistry. All solvents, including anhydrous solvents, and reagents were purchased from Acros Organics, Alfa Aesar, Cayman chemical, Fluka, Sigma-Aldrich or VWR, and used without any further purifications. (3*R*,4*R*)-4-Acetoxy-3-[(*R*)-(tert-butyldimethylsilyloxy)ethyl]-2-azetidinone **7** was obtained from Kaneka corporation (Japan). [^{3}H]-AEA (60 Ci/mmol) and [^{3}H]-2-OG (40 Ci/mmol) were purchased from American Radiolabeled Chemical (St. Louis, MO). UltimaGold scintillation liquid was bought from Perkin-Elmer. All reactions under dry conditions were performed under argon atmosphere in flame-dried glassware. Nuclear magnetic reasonance (^{1}H NMR and ^{13}C NMR) spectra were recorded at 300 MHz for proton and 75 MHz for carbon (Bruker Avance 300) or 500 MHz for proton and 125 MHz for carbon (Bruker Avance 500) using deuterate chloroform ($CDCl_3$). Chemical shifts are reported in ppm relative to the solvent signals ($CDCl_3$ 7.26 and 77.16 ppm). NMR coupling constants (*J*) are reported in hertz. Melting points (mp) were determined on a Büchi B-540 apparatus calibrated with caffeine, vanillin, and phenacetin. Rotations were recorded on Perkin-Elmer 241 MC polarimeter, at 20 °C, in $CHCl_3$. Concentrations are given in percentage (g/100 mL). Low resolution mass spectra were acquired using a Thermo Finnigan LCQ spectrometer in negative mode (ESI). High resolution mass spectrometry (HRMS) analyses were performed at the University of Mons Hainaut (Belgium) or at the University of Oxford (UK). Infrared (IR) spectra were recorded using FTIR-8400S Shimadzu apparatus. Products were analyzed as thin films deposited on a Se−Zn crystal by evaporation from CH_2Cl_2 solutions. Thin layer chromatography (TLC) analysis was performed on Merck silica-gel $60F_{254}$ and detected under UV light, and flash chromatography was performed on silica gel (40−60 mesh) purchased from Rocc (Belgium). Purity of tested compounds was assessed by HPLC on chiral AD-H column (2.1 mm × 150 mm, 5 μm particle size) using hexane/isopropanol eluant (90:10), at a flow rate of 0.5 mL/min and on Symetry C18 (4.6 mm × 250 mm, 5 μm particle size) using acetonitrile/H_2O eluant (70:30), at a flow rate of 1 mL/min (purity ≥97%).

General Procedure for *N*-Acylation. To a stirred solution of azetidinone **7** (1 equiv) in dry dichloromethane (8.6 mL/mmol) at 20 °C were added pyridine (2 equiv) and the suitable acyl chloride (2 equiv) under argon atmosphere. The mixture was refluxed during 24 h, then diluted in dichloromethane and the excess of acyl chloride was quenched by Na_2CO_3 (10% aqueous solution; 8.6 mL/mmol). The organic layer was washed with 3 N HCl and brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. After purification by flash chromatography (cyclohexane/ethyl acetate), white solids (**9a** and **10a−b**) or colorless oils (**9b−c** and **10c−e**) were obtained.

1-(3-Phenylpropanoyl)-(3*R*,4*R*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (9a). Yield: 89% (130.1 mg from 0.35 mmol of **7**). Mp: 70.0−70.5 °C. $[\alpha]_D = -54.0$ (c = 1.0). $R_f = 0.54$ (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 442.1 ($(M + Na)^+$). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.05$ (s, 3H), 0.10 (s, 3H), 0.84 (s, 9H), 1.34 (d, 3H, *J* = 6.4 Hz), 2.14 (s, 3H), 2.97−3.06 (m, 4H), 3.15 (dd, 1H, *J* = 1.5 Hz, *J* = 2.5 Hz), 4.31 (m, 1H), 6.62 (d, 1H, *J* = 1.5 Hz), 7.21−7.34 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -5.3, -4.1$, 17.9, 21.0, 21.9, 25.6, 29.8, 38.3, 64.3, 65.3, 74.3, 126.4, 128.6, 128.6, 140.2, 164.6, 169.1, 169.3. IR (cm^{-1}): υ = 2854−2952, 1803, 1755, 1714, 1454−1495, 1308, 1251, 837. HRMS: C22H33NO5SiNa: calculated: 442.2026, found: 442.2040.

1-(4-Phenylbutanoyl)-(3*R*,4*R*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (9b). Yield: 94% (360 mg from 0.89 mmol of **7**). $[\alpha]_D = -40.2$ (c = 1.0). $R_f = 0.48$ (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 456.2 ($(M + Na)^+$), 888.9 ($(2 M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.03$ (s, 3H), 0.08 (s, 3H), 0.82 (s, 9H), 1.31 (d, 3H, *J* = 6.4 Hz), 1.99 (m, 2H), 2.11 (s, 3H), 2.63−2.78 (m, 4H), 3.12 (m, 1H), 4.29 (m, 1H), 6.59 (d, 1H, *J* = 1.1 Hz), 7.12−7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = -5.3, -4.1$, 17.8, 20.9, 21.9, 25.3, 25.6, 35.1, 35.9, 64.3, 65.1, 74.2, 126.1, 128.4, 128.5, 141.4, 164.5, 169.1, 169.8. IR (cm^{-1}): υ = 2854−2926, 1805, 1757, 1717, 1462, 1308, 1211−1250, 839. HRMS: C23H35NO5SiNa: calculated: 456.2182, found: 456.2187.

1-(Pent-4-enoyl)-(3*R*,4*R*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (9c). Yield: 80% (515 mg from 1.74 mmol of **7**). $R_f = 0.57$ (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 392.1 ($(M + Na)^+$), 760.9 ($(2 M + Na)^+$). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.03$ (s, 3H), 0.07 (s, 3H), 0.82 (s, 9H), 1.31 (d, 3H, *J* = 6.7 Hz), 2.10 (s, 3H), 2.40 (td, 2H, *J* = 7.6 Hz, *J* = 6.5 Hz), 2.74 (td, 1H, *J* = 7.6 Hz, *J* = 16.9 Hz), 2.81 (td, 1H, *J* = 7.6 Hz, *J* = 16.9 Hz), 3.12 (m, 1H), 4.29 (m, 1H), 5.01 (dd, 1H, *J* = 1.6 Hz, *J* = 10.5 Hz), 5.08 (dd, 1H, *J* = 1.6 Hz, *J* = 17.2 Hz), 5.82 (ddt, 1H, *J* = 10.5 Hz *J* = 17.2 Hz, *J* = 6.5 Hz), 6.58 (d, 1H, *J* = 1.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -5.5, -4.3$, 17.6, 20.8, 21.7, 25.5, 27.4, 35.6, 64.1, 65.0, 74.1, 115.8, 136.2, 164.4, 168.9, 169.1. IR (cm^{-1}): υ = 2857−2961, 1808, 1759, 1721, 1642, 1306, 835. HRMS: C18H31NO5SiNa: calculated: 392.1869, found: 392.1863.

1-(3-Phenylpropanoyl)-(3*S*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one (10a). Yield: 97% (617 mg from 1.77 mmol of **8**). $[\alpha]_D = -53.7$ (c = 4.1). $R_f = 0.53$ (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 384.3 ($(M + Na)^+$), 744.9 ($(2 M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.06$ (s, 3H), 0.09 (s, 3H), 0.85 (s, 9H), 1.20 (d, 3H, *J* = 6.3 Hz), 2.95−3.06 (m, 4H), 3.23 (m, 1H), 3.56 (dd, 1H, *J* = 6.7 Hz, *J* = 7.2 Hz), 3.70 (dd, 1H, *J* = 3.6 Hz, *J* = 7.2 Hz), 4.31 (m, 1H), 7.11−7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = -5.1, -4.1$, 17.9, 22.2, 25.7, 30.2, 38.3, 38.4, 56.5, 64.8, 126.3, 128.6 (2C), 140.5, 166.5, 170.2. IR (cm^{-1}): υ = 2856−2955, 1786, 1701, 1310, 1252, 839. HRMS: C20H31NO3SiNa: calculated: 384.1971, found: 384.1974.

1-(4-Phenylbutanoyl)-(3*S*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one (10b). Yield: 87% (286 mg from 0.87 mmol of **8**). Mp: 30.5−31.5 °C. $[\alpha]_D = -42.3$ (c = 1.9). $R_f = 0.52$ (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 376.2 ($(M + H)^+$), 398.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.06$ (s, 3H), 0.09 (s, 3H), 0.85 (s, 9H), 1.20 (d, 3H, *J* = 6.3 Hz), 2.00

(m, 2H), 2.61−2.84 (m, 4H), 3.22 (m, 1H), 3.54 (dd, 1H, J = 6.8 Hz, J = 7.2 Hz), 3.69 (dd, 1H, J = 3.6 Hz, J = 7.2 Hz), 4.31 (m, 1H), 7.14−36 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −5.2, −4.2, 17.8, 22.2, 25.61, 25.66, 35.2, 36.0, 38.2, 56.3, 64.7, 126.0, 128.4, 128.5, 141.5, 166.4, 170.7. IR (cm^{-1}): υ = 2856−3026, 1784, 1697, 1454−1497, 1389, 1309, 1249, 839. HRMS: C21H34NO3Si: calculated: 376.2308, found: 376.2295; C21H33NO3SiNa: calculated: 398.2127, found: 398.2107.

1-(5-Phenylpentanoyl)-(3*S*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one (10c). Yield: 74% (126 mg from 0.43 mmol of **8**). [α]$_D$ = −37.2 (c = 1.0). R_f = 0.53 (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 390.2 ((M + H)$^+$), 412.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.05 (s, 3H), 0.07 (s, 3H), 0.83 (s, 9H), 1.18 (d, 3H, J = 6.3 Hz), 1.56−1.80 (m, 4H), 2.55−2.79 (m, 4H), 3.20 (m, 1H), 3.52 (dd, 1H, J = 6.8 Hz, J = 7.2 Hz), 3.67 (dd, 1H, J = 3.6 Hz, J = 7.2 Hz), 4.30 (m, 1H), 7.07−7.45 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −5.2, −4.2, 17.9, 22.2, 23.7, 25.6, 30.9, 35.6, 36.3, 38.2, 56.3, 64.7, 125.8, 128.3, 128.4, 142.1, 166.4, 170.9. IR (cm^{-1}): υ = 2856−2951, 1784, 1701, 1454−1496, 1389, 1310, 1250, 839. HRMS: C22H36NO3Si: calculated: 390.2464, found: 390.2448; C22H35NO3SiNa: calculated: 412.2284, found: 412.2263.

1-(Pent-4-enoyl)-(3*S*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one (10d). Yield: 95% (965 mg from 3.27 mmol of **8**). R_f = 0.76 (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 312.3 ((M + H)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.03 (s, 3H), 0.05 (s, 3H), 0.82 (s, 9H), 1.17 (d, 3H, J = 6.1 Hz), 2.38 (m, 2H), 2.75 (m, 2H), 3.21 (m, 1H), 3.52 (dd, 1H, J = 6.6 Hz, J = 6.6 Hz), 3.66 (dd, 1H, J = 3.4 Hz, J = 6.6 Hz), 4.29 (m, 1H), 4.97 (dd, 1H, J = 1.6 Hz, J = 10.2 Hz), 5.05 (dd, 1H, J = 1.6 Hz, J = 17.0 Hz), 5.80 (ddt, 1H, J = 6.5 Hz, J = 10.2 Hz, J = 17.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −4.4, −5.4, 17.7, 22.0, 25.4, 27.8, 35.6, 38.1, 56.2, 64.5, 115.5, 136.4, 166.2, 170.1. IR (cm^{-1}): υ = 2858−2930, 1788, 1705, 1311, 840. HRMS: C16H29NO3SiNa: calculated: 334.1814, found: 334.1806.

1-(Hexa-5-enoyl)-(3*S*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one (10e). Yield: 46% (40 mg from 0.27 mmol of **8**). [α]$_D$ = −34.6 (c = 1.0). R_f = 0.60 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 326.2 ((M + H)$^+$), 348.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.05 (s, 3H), 0.07 (s, 3H), 0.83 (s, 9H), 1.19 (d, 3H, J = 6.3 Hz), 1.75 (m, 2H), 2.10 (m, 2H), 2.68 (m, 2H), 3.21 (m, 1H), 3.54 (dd, 1H, J = 6.6 Hz, J = 6.6 Hz), 3.68 (dd, 1H, J = 3.4 Hz, J = 6.6 Hz), 4.30 (m, 1H), 4.96−5.06 (m, 2H), 5.77 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −5.1, −4.1, 18.0, 22.3, 23.3, 25.7, 33.2, 36.0, 38.4, 56.4, 64.8, 115.4, 137.9, 166.5, 171.0. IR (cm^{-1}): υ = 2856−2953, 1786, 1701, 1464, 1389, 1310, 1252, 839. HRMS: C17H31NO3SiNa: calculated: 348.1971, found: 348.1985.

General Procedure for Deprotection. To a stirred suspension of silyl ether (1 equiv) in acetonitrile (30 mL/mmol) at −5 °C was added dropwise 12 N HCl (5 equiv) and 17 N AcOH (7 equiv). The mixture was stirred for 30 min at −5 °C, and for 3 h at 0 °C. Acetonitrile was removed under a vacuum, and the oily residue was diluted in ethyl acetate. The organic layer was washed with 10% $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. After purification by flash chromatography (dichloromethane/ethyl acetate), a white solid (**11a**−**b** and **12a**−**b**) or a colorless oil (**11c**, **12c**−**e**, and **22**) was obtained.

1-(3-Phenylpropanoyl)-(3*R*,4*R*)-3-[1(*R*)-hydroxyethyl]-4-(acetoxy)-azetidin-2-one (11a). Yield: 93% (822 mg from 2.93 mmol of **9a**). Mp: 116.0−117.0 °C. [α]$_D$ = −76.1 (c = 2.9). R_f = 0.20 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 328.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (d, 3H, J = 6.3 Hz), 2.16 (s, 3H), 2.62 (br s, 1H), 2.94−3.16 (m, 4H), 3.17 (dd, 1H, J = 1.4 Hz, J = 6.4 Hz), 4.18 (m, 1H), 6.32 (d, 1H, J = 1.4 Hz), 7.16−7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.0, 21.1, 29.9, 38.4, 64.3, 65.3, 75.9, 126.5, 128.6 (2C), 140.0, 163.2, 169.6, 170.4. IR (cm^{-1}): υ = 3504, 2931−2974, 1803, 1755, 1716, 1454−1496, 1313. HRMS: C16H19NO5Na: calculated: 328.1161, found: 328.1174.

1-(4-Phenylbutanoyl)-(3*R*,4*R*)-3-[1(*R*)-hydroxyethyl]-4-(acetoxy)-azetidin-2-one (11b). Yield: 99% (83 mg from 0.25 mmol of **9b**). Mp: 81.0−81.5 °C. [α]$_D$ = −74.9 (c = 2.9) . R_f = 0.21 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 342.2 ((M + Na)$^+$), 660.77 ((2 M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (d, 3H, J = 6.4 Hz), 2.00 (m, 2H), 2.14 (s, 3H), 2.62 (br s, 1H), 2.64−2.88 (m, 4H), 3.13 (dd, 1H, J = 1.6 Hz, J = 5.7 Hz), 4.21 (m, 1H), 6.27 (d, 1H, J = 1.6 Hz), 7.12−7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.0, 21.1, 25.3, 35.1, 36.0, 64.3, 65.2, 75.9, 126.2, 128.5, 128.6, 141.3, 163.2, 170.2, 170.4. IR (cm^{-1}): υ = 3502, 2931, 1803, 1753, 1716, 1454−1497, 1379, 1308, 1213. HRMS: C17H21NO5Na: calculated: 342.1317, found: 342.1307.

1-(Pent-4-enoyl)-(3*R*,4*R*)-3-[1(*R*)-(hydroxyethyl]-4-(acetoxy)-azetidin-2-one (11c). Yield: 83% (490 mg from 2.3 mmol of **9c**). R_f = 0.40 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 278.1 ((M + Na)$^+$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24 (d, 3H, J = 6.4 Hz), 2.02 (s, 3H), 2.30 (dt, 2H, J = 7.4 Hz, J = 6.8 Hz), 2.70 (m, 1H), 3.08 (dd, 1H, J = 1.4 Hz, J = 5.4 Hz), 3.30 (br s, 1H), 4.13 (qd, 1H, J = 5.4 Hz, J = 6.4 Hz), 4.91 (dd, 1H, J = 1.5 Hz, J = 10.3 Hz), 4.98 (dd, 1H, J = 1.5 Hz, J = 17.1 Hz), 5.72 (ddt, 1H, J = 10.3 Hz, J = 17.1 Hz, J = 6.8 Hz), 6.33 (d, 1H, J = 1.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.7, 20.9, 27.4, 35.6, 63.8, 64.9, 75.2, 115.8, 136.0, 163.3, 169.5, 169.9. IR: υ = 3501, 2932−2978, 1805, 1755, 1718, 1641, 1311. HRMS: C12H17NO5Na: calculated: 278.1004, found: 278.0992.

1-(3-Phenylpropanoyl)-(3*S*)-3-[1(*R*)-hydroxyethyl]-azetidin-2-one (12a). Yield: 78% (297 mg from 1.55 mmol of **10a**). Mp: 64.5−65.5 °C. [α]$_D$ = −36.7 (c = 1.5). R_f = 0.09 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 270.2 ((M + Na)$^+$), 516.9 ((2 M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (d, 3H, J = 6.4 Hz), 1.91 (br s, 1H), 2.88−3.12 (m, 4H), 3.25 (m, 1H), 3.60 (m, 2H), 4.22 (m, 1H), 7.12−7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.7, 30.2, 38.4, 39.2, 55.9, 64.9, 126.4, 128.6, 128.7, 140.4, 166.1, 170.4. IR (cm^{-1}): υ = 3464, 2970, 1782, 1697, 1454−1496, 1387, 1313, 1236. HRMS: C14H17NO3Na: calculated: 270.1106, found: 270.1109.

1-(4-Phenylbutanoyl)-(3*S*)-3-[1(*R*)-hydroxyethyl]-azetidin-2-one (12b). Yield: 86% (165.5 mg from 0.73 mmol of **10b**). Mp: 89.5−90.0 °C. [α]$_D$ = −38.6 (c = 2.5). R_f = 0.10 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 262.1 ((M + H)$^+$), 284.15 ((M + Na)$^+$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.30 (d, 3H, J = 6.4 Hz), 1.68 (br s, 1H), 2.00 (m, 2H) 2.68 (t, 2H, J = 4.5 Hz), 2.73 (t, 2H, J = 4.5 Hz), 3.27 (m, 1H), 3.60 (m, 2H), 4.27 (m, 1H), 7.15−7.30 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 21.7, 25.7, 35.2, 36.1, 39.1, 55.8, 64.9, 126.1, 128.5, 128.6, 141.5, 166.1, 171.0. IR (cm^{-1}): υ = 3449, 2970, 1784, 1697, 1454, 1389, 1312, 1250. HRMS: C15H19NO3Na: calculated: 284.1263, found: 284.1261.

1-(5-Phenylpentanoyl)-(3*S*)-3-[1(*R*)-hydroxyethyl]-azetidin-2-one (12c). Yield: 84% (68.6 mg from 0.30 mmol of **10c**). [α]$_D$ = −13.1 (c = 0.1). R_f = 0.10 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 276.2 ((M + H)$^+$), 298.1 ((M + Na)$^+$). ^{1}H NMR (δ ppm, 300 MHz, $CDCl_3$): δ = 1.27 (d, 3H, J = 6.4 Hz), 1.55−1.80 (m, 4H), 2.49−2.77 (m, 4H), 3.25 (m, 1H), 3.58 (m, 2H), 4.21 (m, 1H), 7.04−7.33 (m, 5H). ^{13}C NMR (δ ppm, 75 MHz, $CDCl_3$): δ = 21.6, 23.7, 30.8, 35.6, 36.4, 39.1, 55.7, 64.7, 125.8, 128.3, 128.4, 142.1, 166.3, 171.2. IR (ATR-SeZn, cm^{-1}): 3445, 2930, 1784, 1697, 1452, 1389, 1312, 1240. HRMS: C16H21NO3Na: calculated: 298.1419, found: 298.1405.

1-(Pent-4-enoyl)-(3*S*)-3-[1(*R*)-hydroxyethyl]-azetidin-2-one (12d). Yield: 85% (457 mg from 2.32 mmol of **10d**). R_f = 0.38 (cyclohexane/ethyl acetate: 5/4). MS (ESI): *m*/*z*: 198.1 ((M + H)$^+$), 220.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (d, 3H, J = 6.4 Hz), 2.38 (td, 2H, J = 7.3 Hz, J = 6.8 Hz), 2.78 (t, 2H, J = 7.3 Hz), 3.28 (m, 1H), 3.56 (br s, 1H), 3.61 (m, 2H), 4.24 (m, 1H), 4.99 (dd, 1H, J = 1.5 Hz, J = 10.3 Hz), 5.72 (dd, 1H, J = 1.5 Hz, J = 17.1 Hz), 5.81 (ddt, 1H, J = 10.3 Hz, J = 17.1 Hz, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.4, 27.8, 35.6, 39.1, 55.7, 64.6, 115.7, 136.3, 166.2, 170.5. IR (cm^{-1}): υ = 3443, 2928−2972, 1786, 1701, 1641, 1315. HRMS: C10H15NO3Na: calculated: 198.1130, found: 198.1122.

1-(Hexa-5-enoyl)-(3*S*)-3-[1(*R*)-hydroxyethyl]-azetidin-2-one (12e). Yield: 94% (20.5 mg from 0.10 mmol of **10e**). $[\alpha]_D$ = −30.2 (c = 3.0). R_f = 0.13 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 212.1 ((M + H)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (d, 3H, *J* = 6.4 Hz), 1.75 (m, 2H), 2.00 (br s, 1H), 2.11 (m, 2H), 2.70 (t, 2H, *J* = 7.5 Hz), 3.27 (m, 1H), 3.61 (d, 2H, *J* = 5.1 Hz), 4.26 (m, 1H), 4.96−5.06 (m, 2H), 5.78 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.7, 23.3, 33.1, 36.0, 39.1, 55.8, 64.9, 115.5, 137.8, 166.2, 171.2. IR (cm^{-1}): v = 3470, 2930, 1786, 1697, 1441−1456, 1389, 1312, 1259. HRMS: C11H17NO3Na: calculated: 212.12866, found: 212.12837.

1-(Benzoyloxycarbonyl)-(3*S*)-3-[1(*R*)-hydroxyethyl]-azetidin-2-one (22). Yield: 91% (98.3 mg from 0.43 mmol of **21**). $[\alpha]_D$ = −37.0 (c = 4.1). R_f = 0.09 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 272.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (d, 3H, *J* = 6.4 Hz), 2.65 (br s, 1H), 3.25 (m, 1H), 3.51−3.75 (m, 2H), 4.20 (m, 1H), 5.22 (s, 2H), 7.21−7.54 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.5, 40.6, 56.8, 64.5, 68.1, 128.4, 128.7 (2C), 135.0, 149.1, 165.9. IR (cm^{-1}): v = 3497, 2972, 1803, 1726, 1456, 1389, 1335. HRMS: C13H15NO4Na: calculated: 272.0899, found: 272.0888.

General Procedure for Esterification with Acyl Chloride (13a−b, 14a−b, 15e, 16a−b, 17a−b, 18b, 19b and 19e, 20b). To a stirred solution of alcohol precursor (1 equiv) in dry dichloromethane (20 mL/mmol), at 20 °C, were added pyridine (2 equiv) and the suitable acyl chloride (2 equiv) under argon atmosphere. After being stirred overnight, the mixture was diluted in dichloromethane and the excess of acyl chloride was quenched by 10% aqueous Na_2CO_3. The organic layer was washed with 3 N HCl and brine, dried over $MgSO_4$, filtered, and concentrated under a vacuum. After purification by flash chromatography (dichloromethane/ethyl acetate), a colorless oil was obtained in all cases.

1-(3-Phenylpropanoyl)-(3*R*,4*R*)-3-[1(*R*)-(3-phenylpropanoyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (13a). Yield: 99% (77 mg from 0.18 mmol of **11a**). $[\alpha]_D$ = −16.6 (c = 5.3). R_f = 0.44 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 460.3 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (d, 3H *J* = 6.9 Hz), 2.15 (s, 3H), 2.64 (m, 2H), 2.90−2.98 (m, 2H), 2.99−3.06 (m, 4H), 3.30 (dd, 1H, *J* = 1.7 Hz, *J* = 5.9 Hz), 5.31 (m, 1H), 6.48 (d, 1H, *J* = 1.7 Hz), 7.17−7.39 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.2, 20.9, 29.8, 30.8, 35.8, 38.3, 62.8, 65.8, 74.8, 126.4, 126.5, 128.3, 128.6, 128.6 (2C), 139.9, 140.2, 162.3, 169.0, 169.3, 171.9. IR (cm^{-1}): v = 2930, 1805, 1736, 1720, 1454, 1381, 1313, 1213. HRMS: C25H27NO6Na: calculated: 460.1736, found: 460.1722.

1-(3-Phenylpropanoyl)-(3*R*,4*R*)-3-[1(*R*)-(4-phenylbutanoyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (13b). Yield: 70% (153 mg from 0.49 mmol of **11a**). $[\alpha]_D$ = −17.7 (c = 2.6). R_f = 0.48 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 474.2 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.41 (d, 3H, *J* = 6.5 Hz), 1.94 (m, 2H), 2.14 (s, 3H), 2.36 (t, 2H, *J* = 7.3 Hz), 2.65 (t, 2H, *J* = 7.6 Hz), 2.93−3.08 (m, 4H), 3.32 (dd, 1H, *J* = 1.6 Hz, *J* = 5.7 Hz), 5.32 (m, 1H), 6.53 (d, 1H *J* = 1.6 Hz), 7.12−7.39 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.1, 20.7, 26.4, 29.7, 33.5, 35.0, 38.2, 62.6, 65.5, 74.6, 126.0, 126.4, 128.4 (2C), 128.5, 128.5, 139.8, 141.2, 162.3, 168.9, 169.2, 172.2. IR (cm^{-1}): v = 2932, 1803, 1742, 1720, 1454, 1381, 1313, 1213, 1188. HRMS: C26H29NO6Na: calculated: 474.1893, found: 474.1893.

1-(4-Phenylbutanoyl)-(3*R*,4*R*)-3-[1(*R*)-(3-phenylpropanoyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (14a). Yield: 88% (90 mg from 0.23 mmol of **11b**). $[\alpha]_D$ = −17.1 (c = 6.2). R_f = 0.42 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 473.9 ((M + Na)$^+$), 925.3 ((2 M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (d, 3H, *J* = 6.5 Hz), 2.00 (m, 2H), 2.12 (s, 3H), 2.57−2.77 (m, 6H), 2.93 (t, 2H, *J* = 7.7 Hz), 3.28 (dd, 1H, *J* = 1.7 Hz, *J* = 5.9 Hz), 5.31 (m, 1H), 6.46 (d, 1H, *J* = 1.7 Hz), 7.14−7.34 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.2, 20.8, 25.3, 30.8, 35.0, 35.7, 36.0, 62.7, 65.8, 74.8, 126.1, 126.4, 128.3 (2C), 128.5, 128.6, 140.2, 141.1, 162.3, 169.0, 169.9, 171.8. IR (cm^{-1}): v = 2935−3028, 1803, 1740, 1717, 1454−1497, 1379, 1310, 1213. HRMS: C26H29NO6Na: calculated: 474.1893, found: 474.1875.

1-(4-Phenylbutanoyl)-(3*R*,4*R*)-3-[1(*R*)-(4-phenylbutanoyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (14b). Yield: 52% (63 mg from 0.26 mmol of **11b**). $[\alpha]_D$ = −11.6 (c = 2.2). R_f = 0.48 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 488.4 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.40 (d, 3H, *J* = 6.5 Hz), 1.81−2.03 (m, 4H), 2.12 (s, 3H), 2.31 (m, 2H), 2.57−2.73 (m, 6H), 3.29 (dd, 1H, *J* = 1.8 Hz, *J* = 5.6 Hz), 5.31 (m, 1H), 6.49 (d, 1H, *J* = 1.8 Hz), 7.22−7.33 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.3, 20.9, 25.4, 26.5, 33.6, 35.0, 35.1, 36.0, 62.7, 65.7, 74.7, 126.1, 126.2, 128.5 (4C), 141.2, 141.3, 162.4, 169.1, 169.9, 172.3. IR (cm^{-1}): v = 2852−3026, 1803, 1736, 1720, 1454−1496, 1381, 1307, 1213, 1058. HRMS: C27H31NO6Na: calculated: 488.2049, found: 488.2044.

1-(Pent-4-enoyl)-(3*R*,4*R*)-3-[1(*R*)-(pent-4-enoyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (15e). Yield: 90% (950 mg from 3.14 mmol of **11c**). $[\alpha]_D$ = −21.9 (c = 5.4). R_f = 0.56 (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 360.0 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39 (d, 3H, *J* = 6.4 Hz), 2.11 (s, 3H), 2.34 (m, 2H), 2.40 (m, 4H), 2.80 (m, 2H), 3.28 (dd, 1H, *J* = 1.6 Hz, *J* = 5.8 Hz), 5.00 (dd, 1H, *J* = 1.6 Hz, *J* = 10.3 Hz), 5.02 (dd, 1H, *J* = 1.6 Hz, *J* = 10.3 Hz), 5.05 (dd, 1H, *J* = 1.6 Hz, *J* = 17.0 Hz), 5.08 (dd, 1H, *J* = 1.6 Hz, *J* = 17.0 Hz), 5.29 (qd, 1H, *J* = 5.8 Hz, *J* = 6.4 Hz), 5.78 (ddt, 1H, *J* = 6.4 Hz, *J* = 10.3 Hz, *J* = 17.0 Hz), 5.82 (ddt, 1H, *J* = 6.4 Hz, *J* = 10.3 Hz, *J* = 17.0 Hz), 6.48 (d, 1H, *J* = 1.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.1, 20.6, 27.4, 28.6, 33.3, 35.7, 62.5, 65.6, 74.5, 115.6, 115.9, 135.9, 136.2, 162.2, 168.8, 169.2, 171.7. IR (cm^{-1}): v = 2982, 1806, 1742, 1722, 1642, 1313. HRMS: C17H23NO6Na: calculated: 360.1423, found: 360.1412.

1-(3-Phenylpropanoyl)-(3*S*)-3-[1(*R*)-(3-phenylpropanoyloxy)-ethyl]-azetidin-2-one (16a). Yield: 79% (64 mg from 0.21 mmol of **12a**). $[\alpha]_D$ = −17.1 (c = 2.7). R_f = 0.41 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 402.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.29 (d, 3H, *J* = 6.4 Hz), 2.61 (t, 2H, *J* = 7.7 Hz), 2.93 (t, 2H, *J* = 7.7 Hz), 2.96−3.08 (m, 4H), 3.31 (m, 1H), 3.41 (dd, 1H, *J* = 3.6 Hz, *J* = 7.7 Hz), 3.56 (dd, 1H, *J* = 6.6 Hz, *J* = 7.7 Hz), 5.21 (m, 1H), 7.09−7.60 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.3, 30.1, 30.9, 35.8, 38.3, 40.1, 53.6, 67.6, 126.4, 126.5, 128.3, 128.56, 128.62 (2C), 140.1, 140.2, 164.3, 170.2, 171.9. IR (cm^{-1}): v = 2931−3028, 1786, 1735, 1701, 1454−1497, 1383, 1315, 1238, 1132−1161. HRMS: C23H25NO4Na: calculated: 402.1681, found: 402.1675.

1-(3-Phenylpropanoyl)-(3*S*)-3-[1(*R*)-(4-phenylbutanoyloxy)-ethyl]-azetidin-2-one (16b). Yield: 75% (79 mg from 0.27 mmol of **12a**). $[\alpha]_D$ = −9.6 (c = 3.4). R_f = 0.44 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 416.2 ((M + Na)$^+$), 808.7 ((2 M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (d, 3H, *J* = 6.4 Hz), 1.95 (m, 2H), 2.32 (t, 2H, *J* = 7.5 Hz), 2.66 (t, 2H, *J* = 7.6 Hz), 2.90−3.11 (m, 4H), 3.41 (m, 1H), 3.32 (dd, 1H, *J* = 3.6 Hz, *J* = 7.7 Hz), 3.68 (dd, 1H, *J* = 6.8, Hz, *J* = 7.7 Hz), 5.28 (m, 1H), 7.12−7.42 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 26.6, 30.1, 33.7, 35.1, 38.3, 40.0, 53.6, 67.3, 126.2, 126.4, 128.5 (2C), 128.6 (2C), 140.2, 141.2, 164.4, 170.2, 172.4. IR (cm^{-1}): v = 2858−3086, 1784, 1732, 1697, 1454−1497, 1381, 1313, 1238, 1132−1190. HRMS: C24H27NO4Na: calculated: 416.1838, found: 416.1827.

1-(4-Phenylbutanoyl)-(3*S*)-3-[1(*R*)-(3-phenylpropanoyloxy)-ethyl]-azetidin-2-one (17a). Yield: 89% (71 mg from 0.20 mmol of **12b**). $[\alpha]_D$ = −9.9 (c = 4.9). R_f = 0.40 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 394.0 ((M + H)$^+$), 416.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31 (d, 3H, *J* = 6.4 Hz), 2.00 (m, 2H), 2.56−2.83 (m, 6H), 2.93 (t, 2H, *J* = 7.6 Hz), 3.33 (m, 1H), 3.41 (dd, 1H, *J* = 3.6, Hz, *J* = 7.7 Hz), 3.56 (dd, 1H, *J* = 6.7, Hz, *J* = 7.7 Hz), 5.24 (m, 1H), 7.01−7.59 (m, 10H).

^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.3, 25.6, 30.9, 35.1, 35.8, 36.0, 40.0, 53.5, 67.6, 126.1, 126.5, 128.3, 128.4, 128.5, 128.6, 140.1, 141.3, 164.3, 170.8, 171.8. IR (cm^{-1}): υ = 2935−3026, 1786, 1736, 1701, 1454−1497, 1383, 1313, 1250, 1132−1190. HRMS: C24H27NO4Na: calculated: 416.1838, found: 416.1821.

1-(4-Phenylbutanoyl)-(3*S*)-3-[1(*R*)-(4-phenylbutanoyloxy)-ethyl]-azetidin-2-one (17b). Yield: 87% (68 mg from 0.19 mmol of **12b**). [α]$_D$ = −3.3 (c = 4.8). R_f = 0.41 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 408.0 ((M + H)$^+$), 430.1 ((M + Na)$^+$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.35 (d, 3H, J = 6.4 Hz), 1.87−2.03 (m, 4H), 2.29 (t, 2H, J = 7.5 Hz), 2.56−2.83 (m, 6H), 3.38 (m, 1H), 3.52 (dd, 1H, J = 3.7 Hz, J = 7.7 Hz), 3.64 (dd, 1H, J = 6.7 Hz, J = 7.7 Hz), 5.28 (m, 1H), 7.12−7.32 (m, 10H). ^{13}C NMR (125 MHz, CDCl$_3$): δ = 18.4, 25.7, 26.6, 33.7, 35.1, 35.2, 36.1, 40.0, 53.6, 67.3, 126.1, 126.2, 128.5, 128.55, 128.57 (2C), 141.2, 141.4, 164.4, 170.9, 172.4. IR (cm^{-1}): υ = 2934, 1786, 1734, 1701, 1454−1497, 1383, 1312, 1246, 1130−1159. HRMS: C25H29NO4Na: calculated: 430.1994, found: 430.1985.

1-(5-Phenylpentanoyl)-(3*S*)-3-[1(*R*)-(4-phenylbutanoyloxy)-ethyl]-azetidin-2-one (18b). Yield: 63% (34 mg from 0.13 mmol of **12c**). [α]$_D$ = −2.2 (c = 1.8). R_f = 0.43 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 444.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.35 (d, 3H, J = 6.3 Hz), 1.57−1.79 (m, 4H), 1.91 (m, 2H), 2.29 (t, 2H, J = 7.5 Hz), 2.54−2.79 (m, 6H), 3.39 (m, 1H), 3.53 (dd, 1H, J = 3.6 Hz, J = 7.5 Hz), 3.65 (dd, 1H, J = 6.8 Hz, J = 7.5 Hz), 5.28 (m, 1H), 7.08−7.38 (m, 10H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.4, 23.7, 26.6, 30.9, 33.7, 35.1, 35.6, 36.5, 39.9, 53.6, 67.3, 125.9, 126.2, 128.4, 128.5, 128.6 (2C), 141.2, 142.1, 164.5, 171.1, 172.4. IR (cm^{-1}): υ = 2858−3026, 1786, 1734, 1699, 1452−1497, 1381, 1313, 1242, 1132−1192. HRMS: C26H31NO4Na: calculated: 444.2151, found: 444.2152.

1-(Pent-4-enoyl)-(3*S*)-3-[1(*R*)-(4-phenylbutanoyloxy)-ethyl]-azetidin-2-one (19b). Yield: 84% (89 mg from 0.31 mmol of **12d**). [α]$_D$ = −1.3 (c = 3.5). R_f = 0.46 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 344.0 ((M + H)$^+$), 366.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.34 (d, 3H, J = 6.4 Hz), 1.92 (m, 2H), 2.30 (t, 2H, J = 7.6 Hz), 2.39 (m, 2H), 2.63 (t, 2H, J = 7.6 Hz), 2.77 (t, 2H, J = 7.4 Hz), 3.40 (m, 1H), 3.53 (dd, 1H, J = 3.7 Hz, J = 7.7 Hz), 3.66 (dd, 1H, J = 6.6 Hz, J = 7.7 Hz), 4.96−5.09 (m, 2H), 5.28 (m, 1H), 5.81 (m, 1H), 7.07−7.36 (m, 5H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.4, 26.5, 27.9, 33.7, 35.0, 35.8, 39.9, 53.6, 67.2, 115.9, 126.1, 128.5 (2C), 136.4, 141.2, 164. 4, 170.3, 172.4. IR (cm^{-1}): υ = 2864−3026, 1786, 1734, 1703, 1454, 1381, 1313, 1238, 1132−1191. HRMS: C20H25NO4Na: calculated: 366.1681, found: 366.1685.

1-(Pent-4-enoyl)-(3*S*)-3-[1(*R*)-(pent-4-enoyloxy)-ethyl]-azetidin-2-one (19e). Yield: 88% (500 mg from 2.03 mmol of **12d**). [α]$_D$ = −0.25 (c = 4.9). R_f = 0.63 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 280.0 ((M + H)$^+$), 302.1 ((M + Na)$^+$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.34 (d, 3H, J = 6.5 Hz), 2.37 (m, 6H), 2.78 (m, 2H), 3.39 (m, 1H), 3.52 (dd, 1H, J = 3.7 Hz, J = 7.8 Hz), 3.65 (dd, 1H, J = 6.5 Hz, J = 7.8 Hz), 4.99 (m, 2H), 5.03 (m, 1H), 5.06 (m, 1H), 5.26 (m, 1H), 5.77 (ddt, 1H, J = 5.9 Hz, J = 10.2 Hz, J = 16.2 Hz), 5.81 (ddt, 1H, J = 6.5 Hz, J = 10.2 Hz, J = 16.8 Hz). ^{13}C NMR (125 MHz, CDCl$_3$): δ = 18.2, 27.8, 28.7, 33.4, 35.7, 39.8, 53.4, 67.2, 115.6, 115.7, 136.1, 136.2, 164.2, 170.2, 171.7. IR (cm^{-1}): υ = 2927−2979, 1785, 1738, 1702, 1320. HRMS: C15H21NO4Na: calculated: 302.1368, found: 302.1358.

1-(Hexa-5-enoyl)-(3*S*)-3-[1(*R*)-(4-phenylbutanoyloxy)-ethyl]-azetidin-2-one (20b). Yield: 77% (20 mg from 0.07 mmol of **12e**). [α]$_D$ = −2.6 (c = 1.0). R_f = 0.43 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 358.0 ((M + H)$^+$), 380.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.35 (d, 3H, J = 6.4 Hz), 1.74 (m, 2H), 1.92 (m, 2H), 2.10 (m, 2H), 2.29 (t, 2H, J = 7.5 Hz), 2.56−2.74 (m, 4H), 3.39 (m, 1H), 3.53 (dd, 1H, J = 3.7 Hz, J = 7.7 Hz), 3.66 (dd, 1H, J = 6.6 Hz, J = 7.7 Hz), 4.93−5.02 (m, 2H), 5.28 (m, 1H), 5.77 (m, 1H), 7.10−7.36 (m, 5H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.4, 23.2, 26.6, 33.1, 33.7, 35.1, 35.9, 40.0, 53.6, 67.3, 115.6, 126.2, 128.6 (2C), 137.7, 141.2, 164.5, 171.1, 172.4. IR (cm^{-1}): υ = 2934−2976, 1786, 1734, 1701, 1454, 1381, 1313, 1250, 1132−1190. HRMS: C21H27NO4Na: calculated: 380.1838, found: 380.1827.

General Procedure for Esterification with Carboxylic Acid (16c−d, 17c−d, 18d, 19d and 19f, 20d and 23). To a stirred solution of alcohol precursor, DCC (1.1 equiv) and DMAP (cat.) in dry dichloromethane (13 mL/mmol), at 20 °C, was added a solution of the suitable carboxylic acid (1.1 equiv) in dry dichloromethane (7 mL/mmol) under argon atmosphere. After stirring overnight, the mixture was cooled in an ice-bath for precipitation of urea, filtered, and concentrated under a vacuum. After purification by flash chromatography (dichloromethane/ethyl acetate), white solids (**13d**, **14d**, **16d**, **17d**, **18d**, **19d**, **20d**, and **23**) or colorless oils (**16c**, **17c**, and **19f**) were obtained.

1-(3-Phenylpropanoyl)-(3*R*,4*R*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (13d). Yield: 67% (35 mg from 0.16 mmol of **11a**). Mp: 98.0−103.0 °C. [α]$_D$ = −15.1 (c = 2.3). R_f = 0.28 (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 522.2 ((M + Na)$^+$), 1020.9 ((2 M + Na)$^+$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.42 (d, 3H, J = 6.5 Hz), 2.14 (s, 3H), 2.76−2.94 (m, 4H), 3.29 (dd, 1H, J = 1.7 Hz, J = 5.6 Hz), 3.64 (d, 1H, J = 17.1 Hz, AB system), 3.70 (d, 1H, J = 17.1 Hz, AB system), 5.33 (m, 1H), 6.46 (d, 1H, J = 1.7 Hz), 7.13−7.66 (m, 14H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.3, 20.9, 29.8, 38.3, 41.0, 62.8, 66.2, 74.8, 126.5, 127.2, 127.4 (2C), 128.6, 128.6, 128.9, 129.8, 132.6, 140.0, 140.2, 140.7, 162.2, 169.1, 169.2, 170.5. IR (cm^{-1}): υ = 2931−3029, 1803, 1740, 1718, 1454−1489, 1381, 1313, 1213. HRMS: C30H29NO6Na: calculated: 522.1893, found: 522.1899.

1-(4-Phenylbutanoyl)-(3*R*,4*R*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-4-(acetoxy)-azetidin-2-one (14d). Yield: 77% (62 mg from 0.16 mmol of **11b**). Mp: 87.5−89.0 °C. [α]$_D$ = −8.2 (c = 4.7). R_f = 0.32 (cyclohexane/ethyl acetate: 5/2). MS (ESI): *m*/*z*: 536.1 ((M + Na)$^+$), 1048.6 ((2 M + Na)$^+$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.43 (d, 3H, J = 6.5 Hz), 1.85−2.00 (m, 2H), 2.12 (s, 3H), 2.48−2.73 (m, 4H), 3.28 (dd, 1H, J = 1.7 Hz, J = 5.6 Hz), 3.64 (d, 1H, J = 15.5 Hz, AB system), 3.70 (d, 1H, J = 15.5 Hz, AB system), 5.35 (m, 1H), 6.46 (d, 1H, J = 1.7 Hz), 7.15−7.70 (m, 14H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.3, 20.9, 25.2, 35.0, 36.0, 41.0, 62.7, 66.2, 74.7, 126.1, 127.1, 127.4 (2C), 128.5 (2C), 128.9, 129.7, 132.6, 140.2, 140.7, 141.2, 162.2, 169.1, 169.8, 170.4. IR (cm^{-1}): υ = 2854−3082, 1803, 1740, 1717, 1452−1489, 1381, 1312, 1213. HRMS: C31H31NO6Na: calculated: 536.2049, found: 536.2062.

1-(3-Phenylpropanoyl)-(3*S*)-3-[1(*R*)-(5-phenylpentanoyloxy)-ethyl]-azetidin-2-one (16c). Yield: 59% (48 mg from 0.20 mmol of **12a**). [α]$_D$ = −9.9 (c = 1.8). R_f = 0.44 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 430.1 ((M + Na)$^+$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.35 (d, 3H, J = 6.5 Hz), 1.58−1.76 (m, 4H), 2.32 (m, 2H), 2.63 (m, 2H), 2.96−3.09 (m, 4H), 3.38 (m, 1H), 3.52 (dd, 1H, J = 3.6 Hz, J = 7.7 Hz), 3.66 (dd, 1H, J = 6.5 Hz, J = 7.7 Hz), 5.25 (m, 1H), 7.14−7.40 (m, 10H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.4, 24.6, 30.2, 30.9, 34.3, 35.6, 38.3, 40.1, 53.6, 67.3, 125.9, 126.4, 128.5 (2C), 128.6 (2C), 140.2, 142.0, 164.4, 170.3, 172.5. IR (cm^{-1}): υ = 2932−3026, 1785, 1734, 1701, 1454−1497, 1387, 1315, 1238−1255, 1132−1175. HRMS: C25H29NO4Na: calculated: 430.1994, found: 430.1990.

1-(3-Phenylpropanoyl)-(3*S*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-azetidin-2-one (16d). Yield: 93% (84 mg from 0.20 mmol of **12a**). Mp: 61.5−62.0 °C. [α]$_D$ = −18.2 (c = 3.7). R_f = 0.38 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 464.2 ((M + Na)$^+$), 904.8 ((2 M + Na)$^+$). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.29 (d, 3H, J = 6.4 Hz), 2.77−2.96 (m, 4H), 3.31 (m, 1H), 3.42 (dd, 1H, J = 3.7 Hz, J = 7.7 Hz), 3.54 (dd, 1H, J = 7.2 Hz, J = 7.7 Hz), 3.58 (s, 2H), 5.22 (m, 1H), 7.09−7.60 (m, 14H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 18.5, 30.2, 38.4, 39.9, 41.3, 53.7, 67.9, 126.5, 127.3, 127.5 (2C), 128.7 (2C), 129.0, 129.7, 132.8, 140.3, 140.4, 140. 7, 164.3, 170.3, 170.5. IR (cm^{-1}): v = 2906−3058, 1786, 1734, 1701, 1454−1489, 1387, 1315, 1251, 1132−1157. HRMS: C28H27NO4Na: calculated: 464.1838, found: 464. 1845.

1-(4-Phenylbutanoyl)-(3*S*)-3-[1(*R*)-(5-phenylpentanoyloxy)-ethyl]-azetidin-2-one (17c). Yield: 93% (76 mg from 0.19 mmol of **12b**). $[\alpha]_{20/D}$ = −2.3 (c = 4.0). R_f = 0.41 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 421.9 ($(M + H)^+$), 444.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.35 (d, 3H, *J* = 6.4 Hz), 1.56−1.73 (m, 4H), 2.01 (m, 2H), 2.31 (m, 2H), 2.62 (m, 2H), 2.66−2.84 (m, 4H), 3.38 (m, 1H), 3.51 (dd, 1H, *J* = 3.7 Hz, *J* = 7.7 Hz), 3.63 (dd, 1H, *J* = 6.6 Hz, *J* = 7.7 Hz), 5.27 (m, 1H), 7.08−7.45 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 24.6, 25.7, 30.8, 34.2, 35.1, 35.5, 36.0, 39.9, 53.5, 67.2, 125.9, 126.1, 128.4 (2C), 128.5 (2C), 141.3, 142.0, 164.4, 170.8, 172.5. IR (cm^{-1}): v = 2856−3026, 1786, 1734, 1701, 1454, 1383, 1313, 1250, 1130. HRMS: C26H31NO4Na: calculated: 444.2151, found: 444.2141.

1-(4-Phenylbutanoyl)-(3*S*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-azetidin-2-one (17d). Yield: 83% (72 mg from 0.19 mmol of **12b**). Mp: 96.0−96.5 °C. $[\alpha]_D$ = −14.8 (c = 4.7). R_f = 0.40 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 456.0 ($(M + H)^+$), 478.1 ($(M + Na)^+$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.37 (d, 3H, *J* = 6.4 Hz), 1.95 (m, 2H), 2.57−2.76 (m, 4H), 3.37 (m, 1H) 3.48 (dd, 1H, *J* = 3.7 Hz, *J* = 7.7 Hz), 3.59 (dd, 1H, *J* = 6.6 Hz, *J* = 7.7 Hz), 3.65 (s, 2H), 5.31 (m, 1H), 6.98−7.76 (m, 14H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.4, 25.5, 35.1, 36.0, 39.7, 41.1, 53.5, 67.8, 126.1, 127.1, 127.38, 127.43, 128.4, 128.5, 128.9, 129.6, 132.6, 140.2, 140.6, 141.3, 164.2, 170.4, 170.7. IR (cm^{-1}): v = 2936−3028, 1786, 1736, 1697, 1452−1489, 1389, 1313, 1248, 1132−1155. HRMS: C29H29NO4Na: calculated: 478.1994, found: 478.1994.

1-(5-Phenylpentanoyl)-(3*S*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-azetidin-2-one (18d). Yield: 66% (39 mg from 0.12 mmol of **12c**). Mp: 70.5−71.3 °C. $[\alpha]_D$ = −1.1 (c = 7.0). R_f = 0.44 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 492.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (d, 3H, *J* = 6.4 Hz), 1.52−1.73 (m, 4H), 2.50−2.75 (m, 4H), 3.38 (m, 1H), 3.48 (dd, 1H, *J* = 3.6 Hz, *J* = 7.7 Hz), 3.56−3.65 (m, 3H), 5.31 (m, 1H), 7.07−7.64 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 23.7, 30.8, 35.6, 36.4, 39.7, 41.2, 53.5, 67.8, 125.8, 127.1, 127.4, 127.5, 128.4, 128.5, 128.9, 129.6, 132.7, 140.2, 140.6, 142.1, 164.3, 170.4, 170.9. IR (cm^{-1}): v = 2854−3028, 1786, 1736, 1699, 1452−1489, 1389, 1315, 1246, 1132−1159. HRMS: C30H31NO4Na: calculated: 492.2151, found: 492.2133.

1-(Pent-4-enoyl)-(3*S*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-azetidin-2-one (19d). Yield: 68% (70 mg from 0.26 mmol of **12d**). Mp: 92.5−93.0 °C. $[\alpha]_D$ = −9.8 (c = 2.5). R_f = 0.44 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 414.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (d, 3H, *J* = 6.4 Hz), 2.36 (m, 2H), 2.70 (m, 2H), 3.39 (m, 1H), 3.48 (dd, 1H, *J* = 3.7 Hz, *J* = 7.7 Hz), 3.61 ((dd, 1H, *J* = 6.6 Hz, *J* = 7.7 Hz), 3.64 (s, 2H), 5.00 (m, 2H), 5.30 (m, 1H), 5.79 (m, 1H), 7.27−7.70 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 27.9, 35.8, 39.8, 41.2, 53.6, 67.8, 115.8, 127.1, 127.4, 128.9 (2C), 129.6, 132.7, 136.4, 140.2, 140.6, 164.3, 170.3, 170.4. IR (cm^{-1}): v = 2916, 1788, 1734, 1701, 1488, 1387, 1315, 1238−1259. HRMS: C24H25NO4Na: calculated: 414.1681, found: 414.1692.

1-(Pent-4-enoyl)-(3*S*)-3-[1(*R*)-(hexa-5-enoyloxy)-ethyl]-azetidin-2-one (19f). Yield: 84% (65 mg from 0.26 mmol of **12d**). $[\alpha]_D$ = −0.5 (c = 4.0). R_f = 0.41 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 316.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32 (d, 3H, *J* = 6.4 Hz), 1.66 (m, 2H), 2.04 (m, 2H), 2.26 (t, 2H, *J* = 7.5 Hz), 2.38 (m, 2H), 2.76 (t, 2H, *J* = 7.5 Hz), 3.39 (m, 1H), 3.51 (dd, 1H, *J* = 3.6 Hz, *J* = 7.7 Hz), 3.64 (dd, 1H, *J* = 6.8 Hz, *J* = 7.7 Hz), 4.85−5.12 (m, 4H), 5.22 (m, 1H), 5.76 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 24.1, 28.0, 33.0, 33.6, 35.9, 39.9, 53.6, 67.2, 115.7, 115.9, 136.4, 137.5, 164.5, 170.4, 172.5. IR (cm^{-1}): v = 2935−2978, 1788, 1736, 1701, 1381, 1315, 1238, 1134−1168. HRMS: C16H23NO4Na: calculated: 316.1525, found: 316.1515.

1-(Hexa-5-enoyl)-(3*S*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-azetidin-2-one (20d). Yield: 66% (39 mg from 0.06 mmol of **12e**). Mp: 44.2−45.1 °C. $[\alpha]_D$ = −11.7 (c = 1.1). R_f = 0.43 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 428.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (d, 3H, *J* = 6.4 Hz), 1.71 (m, 2H), 2.06 (m, 2H), 2.61 (m, 2H), 3.39 (m, 1H), 3.49 (dd, 1H, *J* = 3.7 Hz, *J* = 7.7 Hz), 3.61 (dd, 1H, *J* = 7.2 Hz, *J* = 7.7 Hz), 3.64 (s, 2H), 4.90−5.06 (m, 2H), 5.30 (m, 1H), 5.74 (m, 1H), 7.23−7.65 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 23.2, 33.0, 35.9, 39.8, 41.2, 53.5, 67.8, 115.5, 127.1, 127.4 (2C), 128.9, 129.6, 132.7, 137.7, 140.3, 140.6, 164.3, 170.5, 171.0. IR (cm^{-1}): v = 2934−2976, 1786, 1736, 1701, 1450−1489, 1389, 1315, 1252, 1132−1194. HRMS: C25H27NO4Na: calculated: 428.1838, found: 428.1825.

1-(Benzyloxycarbonyl)-(3*S*)-3-[1(*R*)-(biphenylacetyloxy)-ethyl]-azetidin-2-one (23). Yield: 83% (75 mg from 0.20 mmol of **22**). Mp: 90.8−91.6 °C. $[\alpha]_D$ = −19.8 (c = 0.6). R_f = 0.37 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 466.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.35 (d, 3H, *J* = 6.3 Hz), 3.39 (m, 1H), 3.51 (dd, 1H, *J* = 3.6 Hz, *J* = 7.0 Hz), 3.60−3.70 (m, 3H), 5.19 (s, 2H), 5.29 (m, 1H), 7.26−7.67 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.4, 41.2, 41.4, 54.5, 68.0, 68.2, 127.2 (2C), 128.4 (2C), 128.7, 128.8, 128.9, 129.7, 132.7, 135.0, 140.2, 140.8, 148.9, 163.6, 170.6. IR (cm^{-1}): 2920−3059, 1813, 1772, 1730, 1456−1489, 1389, 1329, 1128. HRMS: C27H25NO5Na: calculated: 466.1630, found: 466.1609.

1-(Benzyloxycarbonyl)-(3*S*)-3-[1(*R*)-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one (21). To a stirred solution of lithium hexamethyldisilazide (436 μL, 0.44 mmol) in tetrahydrofurane (2 mL) at −78 °C was added **8** (100 mg, 0.44 mmol) in tetrahydrofurane (2 mL) under argon atmosphere. The mixture was stirred for 30 min at −78 °C; then benzyl chloroformate (75 μL, 0.52 mmol) was added. After being stirred for 1 h, at low temperature, the solution was allowed to warm up and was stirred for 1 h at 20 °C. After dilution in dichloromethane, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under a vacuum. Purification by flash chromatography (cyclohexane/ethyl acetate) gave **21** as a white solid. Yield: 99% (157 mg from 0.44 mmol of **8**). Mp: 44.6−46.0 °C. R_f = 0.46 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 386.1 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.03 (s, 3H), 0.06 (s, 3H), 0.81 (s, 9H), 1.17 (d, 3H, *J* = 6.3 Hz), 3.22 (m, 1H), 3.58 (dd, 1H, *J* = 3.5 Hz, *J* = 6.5 Hz), 3.73 (dd, 1H, *J* = 6.4 Hz, *J* = 6.5 Hz), 4.29 (m, 1H), 5.25 (s, 2H), 7.27−7.48 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = −5.1, −4.1, 17.9, 22.3, 25.7, 39.8, 57.3, 64.8, 68.0, 128.4, 128.5, 128.7, 135.4, 149.3, 165.8. IR (cm^{-1}): v = 2854−2924, 1801, 1726, 1464, 1387, 1323−1339, 1259. HRMS: C19H29NO4SiNa: calculated: 386.1764, found: 386.1776.

(3*S*)-3-[1(*R*)-(Biphenylacetyloxy)-ethyl]-azetidin-2-one (24). To a stirred solution of **23** (56 mg, 0.13 mmol) in ethyl acetate (2.5 mL) and ethanol (3 mL) was added 10% Pd/C (5.6 mg). After being stirred under hydrogen atmosphere (*P* = 1 atm), during 1 h at room temperature, the mixture was filtered through a short pad of Celite and concentrated under a vacuum. **24** was obtained without further purification as a white solid. Yield: 96% (38 mg from 0.13 mmol of **23**). Mp: 119.5−120.8 °C. $[\alpha]_D$ = −27.2 (c = 2.0). R_f = 0.06 (cyclohexane/ethyl acetate: 5/3). MS (ESI): *m*/*z*: 309.8 ($(M + H)^+$), 332.0 ($(M + Na)^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (d, 3H, *J* = 6.3 Hz), 3.14 (dd, 1H, *J* = 2.3 Hz, *J* = 5.5 Hz), 3.32 (dd, 1H, *J* = 5.4 Hz, *J* = 5.5 Hz), 3.35−3.43 (m, 1H), 3.65 (s, 2H), 5.25 (m, 1H), 5.96 (br s, 1H), 7.36 (m, 3H), 7.44 (m, 2H), 7.56 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.5, 39.3, 41.2, 56.5, 69.1, 127.1, 127.3, 127.4, 128.9, 129.7, 133.0, 140.1, 140.7, 167.7, 170.8. IR (cm^{-1}): v = 3248, 2922−2978, 1755, 1732, 1489, 1250, 1136−1155. HRMS: C19H19NO3Na: calculated: 332.1263, found: 332.1249.

In Vitro Assays for Human FAAH. Tubes containing the enzyme[47] (10 mM Tris-HCl, 1 mM EDTA, 0.1% (w/v) BSA, pH 7.4, 165 μL), test compounds in DMSO or DMSO alone for controls (10 μL) and [^{3}H]-AEA (50 000 dpm, 2 μM final concentration, 25 μL) were incubated at 37 °C for 10 min. Reactions were stopped by rapidly placing the tubes in ice and adding 400 μL of ice-cold chloroform/methanol (1:1 v/v) followed by vigorous mixing. Phases were separated by centrifugation at 850*g*, and aliquots (200 μL) of the upper methanol/buffer phase were counted for radioactivity by liquid scintillation counting. In all experiments, tubes containing buffer only were used as control for chemical hydrolysis (blank) and this value was systematically subtracted. Using these conditions, URB-597 inhibits *h*FAAH with an IC$_{50}$ value of 40 nM.

In Vitro Assays for Human MGL Activity. Tubes containing purified enzyme[46] (10 mM Tris-HCl, 1 mM EDTA, 0.1% (w/v) BSA, pH 8.0, 165 μL), test compounds in DMSO or DMSO alone for controls (10 μL) and [^{3}H]-2-OG (50 000 dpm, 2 μM final concentration, 25 μL) were incubated at 37 °C for 10 min. Reactions were stopped by rapidly placing the tubes in ice and adding 400 μL of ice-cold chloroform/methanol (1:1 v/v) followed by vigorous mixing. Phases were separated by centrifugation at 850*g*, and aliquots (200 μL) of the upper methanol/buffer phase were counted for radioactivity by liquid scintillation counting. In all experiments, tubes containing buffer only were used as control for chemical hydrolysis (blank) and this value was systematically subtracted.

Preincubation Studies. Tubes containing enzyme (10 mM Tris-HCl, 1 mM EDTA, 0.1% (w/v) BSA, pH 7.4, 165 μL) and test compounds in DMSO or DMSO alone (10 μL) were preincubated 90, 45, 15, and 0 min at room temperature prior to addition of [^{3}H]-AEA (50 000 dpm, 2 μM final concentration, 25 μL). Reactions were stopped by rapidly placing the tubes in ice and adding 400 μL of ice-cold chloroform/methanol (1:1 v/v) followed by vigorous mixing. Phases were separated by centrifugation at 850*g*, and aliquots (200 μL) of the upper methanol/buffer phase were counted for radioactivity by liquid scintillation counting. In all experiments, tubes containing buffer only were used as control for chemical hydrolysis (blank) and this value was systematically subtracted.

Reversibility Studies. In a total volume of 15 μL, human FAAH (27.5 μg) and inhibitors (or DMSO for controls) at concentrations allowing inhibition of the enzyme before dilution and no inhibition after the 100-fold dilution were preincubated during 1 h at room temperature. The mixtures were then diluted 100-fold by adding assay buffer. Immediately after, an aliquot (165 μL) was taken and [^{3}H]-AEA (50 000 dpm, 2 μM final concentration, 25 μL) was added. Two samples were taken at 30 and 90 min after the dilution too. Each aliquots were incubated at 37 °C for 30 min and reactions were stopped by rapidly placing the tubes in ice and adding 400 μL of ice-cold chloroform/methanol (1:1 v/v) followed by vigorous mixing. Phases were separated by centrifugation at 850*g*, and aliquots (200 μL) of the upper methanol/buffer phase were counted for radioactivity by liquid scintillation counting. In all experiments, tubes containing buffer only were used as control for chemical hydrolysis (blank) and this value was systematically subtracted.

Determination of Inhibitor Interactions with *h*FAAH. Tubes containing enzyme (10 mM Tris-HCl, 1 mM EDTA, 0.1% (w/v) BSA, pH 7.4, 165 μL; except for 150 μM of AEA, 159.5 μL and 250 μM of AEA, 139.5 μL) and test compounds in DMSO or DMSO alone (10 μL) were incubated at 37 °C with increasing concentrations of [^{3}H]-AEA (50,000 dpm, 1, 2, 5, 10, 15, 20, 30, 75, 150, and 250 μM final concentration, 25 μL; except for 150 μM, 30.5 μL and 250 μM, 50.5 μL). Reactions were stopped by rapidly placing the tubes in ice and adding 400 μL of ice-cold chloroform/methanol (1:1 v/v) followed by vigorous mixing. Phases were separated by centrifugation at 850 g, and aliquots (200 μL) of the upper methanol/buffer phase were counted for radioactivity by liquid scintillation counting. In all experiments, tubes containing buffer only were used as control for chemical hydrolysis (blank) and this value was systematically subtracted.

Docking Studies. Docking of the inhibitors into the active site of FAAH was performed using the GOLD program. GOLD is based on a genetic algorithm, performing docking of flexible ligands into proteins with partial flexibility in the neighborhood of the active site. Default settings were used for the genetic algorithm parameters. Twenty solutions were generated and ranked by GOLD score. The GOLD fitness function is made up of four components: protein−ligand hydrogen bond energy, protein−ligand van der Walls energy, ligand internal van der Waals energy, and ligand torsional strain energy. The figures were produced using PyMOL[57] and Ligplot.[58]

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Supporting Information Available: Synthesis of compound **8**, pI$_{50}$ and standard deviation of each tested compound, representative "dose−response" curves, docking showing aminoacids involved in hydrophobic contacts and Ramachadran plot of the modeled human FAAH. This material is available free of charge via the Internet at http://pubs.acs.org.

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