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Synthesis and Antibiofilm Activity of a Second-Generation Reverse-Amide Oroidin Library: A Structure–Activity Relationship Study

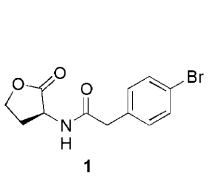
T. Eric Ballard, Justin J. Richards, Amanda L. Wolfe, and Christian Melander*[a]

Abstract: A second-generation library of 2-aminoimidazole-based derivatives incorporating a "reversed amide" (RA) motif in comparison to the marine natural product oroidin were synthesized and subsequently assayed for antibiofilm activity against the medically relevant Gram-negative proteobacteria *P. aeruginosa* and *A. baumannii*. Most notably, an in-depth activity profile is reported for the most active subclass of derivatives that bear linear aliphatic chains off the amide bond. Additionally, further structural modifications of the core template, such as removal of the amide bond or substitution with a triazole isostere, resulted in the discovery of analogues with antibiofilm activities that varied with respect to their inhibition and dispersal properties of *P. aeruginosa* and *A. baumannii* biofilms.

Keywords: amino acids · biological activity · drug design · medicinal chemistry · structure–activity relationships

Introduction

It has been estimated that approximately 80% of the world's microbial biomass resides in the biofilm state and yet there are relatively few known naturally occurring molecular scaffolds that possess the ability to disrupt biofilm development and maintenance (e.g., **1**–**3**).[1–4] Bacterial biofilms are often described as surface-associated complex communities of bacteria encased in a protective extracellular matrix.[5] Pathogenic infections commonly persist due to the respective bacteria's ability to form robust biofilms, which are much less susceptible to traditional means of eradication including antiseptics and antibiotic therapy.[5,6] Additionally, given the combination of high morbidity and mortality rates of infectious diseases due to biofilm virulence, there is a significant need for new antibiofilm modulators.



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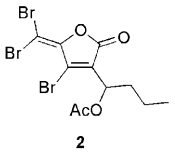

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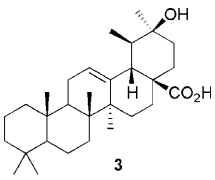

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Antibiotic drug resistance is a matter of survival for bacteria and has been problematic since the beginning of heavy administration of antibiotics in the mid 1900s. With the introduction of penicillin, only several years were needed for resistant bacterial strains to appear.[7,8] Today, multidrug resistant bacteria are becoming increasingly commonplace and various antibiotics are being exhausted in attempts to combat particularly aggressive infections.[8–10] Additionally, only two new classes of antibiotics (oxazolidinones and lipopeptides) have been discovered in the last 40 years.[11] Vancomycin and its congeners remain at the forefront of antibiotic therapy, but even vancomycin-resistant strains are beginning to appear.[8,12]

As most pathogenic bacteria exist as biofilm communities, which hinder antibiotic effectiveness and facilitate the evolution of resistant phenotypes,[5,6] the discovery and development of antibiofilm-modulating compounds should have an impact on human medicine.[13] Given that antibiofilm modulators inhibit and/or disperse bacterial biofilms in a nontoxic manner, there is significant potential for co-dosing antibiofilm compounds with an antibiotic to eliminate biofilm infections. This approach may be particularly important to treat cystic fibrosis (CF) patients, infection of indwelling medical devices, and the remediation of hospital facilities. Furthermore, the lack of induced microbicidal activity

[a] Dr. T. E. Ballard,[+] J. J. Richards,[+] A. L. Wolfe, Prof. Dr. C. Melander
Department of Chemistry
North Carolina State University
Raleigh, NC 27695-8204 (USA)
Fax: (+1) 919-515-5079
E-mail: christian_melander@ncsu.edu

[+] Both authors contributed equally to this work.

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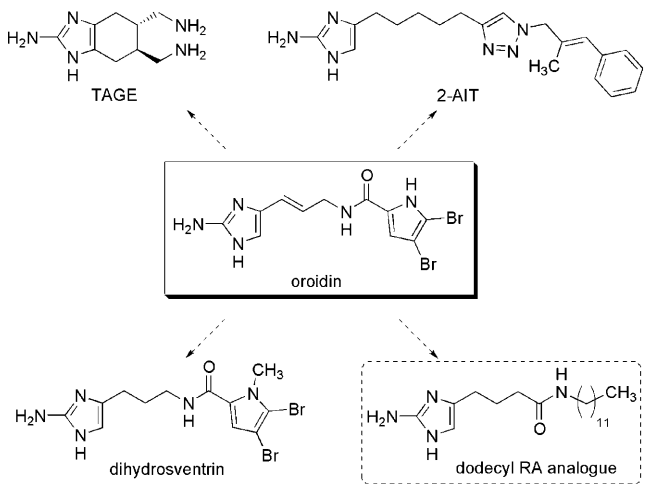
should limit or mitigate development of resistance to the antibiofilm molecule.

Our research[14–20] has focused on the generation of small-molecule libraries that borrow structural inspiration from the oroidin family[21] of marine natural products. We have been able to identify effective antibiofilm modulators by exploiting the oroidin scaffold against a number of medically relevant biofilm-forming bacteria (Scheme 1).[14–20] Most notably, the reverse-amide (RA) motif has been one of the most potent structure classes formulated to date.[17] After the preliminary success of our pilot RA library, delineation of a detailed structure–activity relationship (SAR) study was deemed as an attractive approach to generate antibiofilm compounds with superior activity.



Scheme 1. Oroidin as inspiration for novel classes of biofilm modulators.

Previously, the first-generation RA library was screened against PAO1 and PA14, two of the most commonly studied strains of the bacterium *Pseudomonas aeruginosa. P. aeruginosa* is an opportunistic Gram-negative γ-proteobacteria that is relatively innocuous to healthy individuals and is ubiquitous throughout the environment.[22] However, *P. aeruginosa* is the second most common pathogenic bacteria in hospital-acquired pneumonia and is a serious threat to cystic fibrosis (CF) patients.[23–27] A combination of genetic disposition and the abnormal composition of respiratory airways in CF patients in conjunction with the virulence of *P. aeruginosa* commonly leads to chronic infections that drive increased morbidity and mortality rates.[28] The inability to treat CF patients with chronic *P. aeruginosa* infections has been directly correlated to the virulence of *P. aeruginosa* biofilms.[24,26]

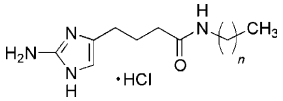
Another opportunistic γ-proteobacterium that has become a serious threat over the last decade due to its multidrug resistance is *Acinetobacter baumannii*.[29] This Gram-negative bacterium is also benign to healthy individuals, but onset of an *A. baumannii* infection can be life-threatening. *A. baumannii* biofilms are particularly hardy, being able to survive for weeks on inanimate objects, thus making eradication especially problematic.[30] Approximately 25% of all hospital swabs are positive for *A. baumannii*.[31] Reports of antibiotic resistance to carbapenems[32] and polymyxins[33] are becoming increasingly frequent. It is believed that this is attributed to changes in porin proteins, development of efflux pumps, and production of β-lactamases in the bacteria.[26,32] Biofilms easily facilitate these adaptations for survival through the selection and reproduction of bacteria that can withstand harsh environmental pressures. Only a few small molecules have been documented to modulate the biofilm activity of *A. baumannii* and these have all originated from the oroidin scaffold.[14,18–20] Therefore, screening our second-generation RA library for inhibition and dispersion activity against *A. baumannii* biofilms was also pursued.

Herein, we report the synthesis of a diverse 24-compound second-generation RA library and its subsequent antibiofilm properties against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Analysis of the activity trends points to important structural features that augment antibiofilm properties.

Results and Discussion

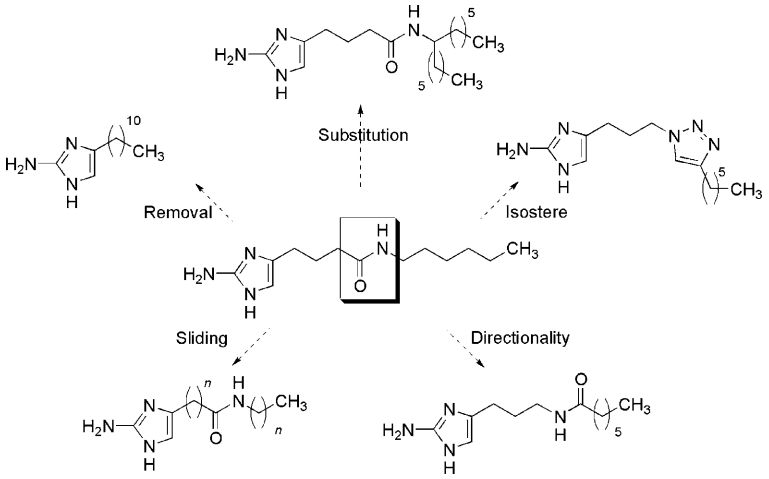
Design: The first-generation RA library identified the dodecyl-based RA analogue **7** (Table 1) as being both a potent inhibitor and disruptor of *P. aeruginosa* biofilms (PA14 biofilm inhibition $IC_{50}=(2.3\pm0.83)$ μM, PA14 biofilm dispersion $EC_{50}=(21\pm3.9)$ μM).[17] However, a more comprehensive study was initiated to identify the optimal chain length for antibiofilm potency (Table 1).

Table 1. Examining the aliphatic chain length.



1st generation	Proposed 2nd generation
4 ($n=5$)	**8** ($n=10$)
5 ($n=7$)	**9** ($n=12$)
6 ($n=9$)	**10** ($n=13$)
7 ($n=11$)	**11** ($n=15$)
	12 ($n=17$)

Following the completion of the chain-length study, our design for a second-generation RA library would be roughly based on derivatization around the hexyl RA analogue **4**, the synthesis and activity of which was also previously disclosed.[17] This analogue was chosen as a template for additional SAR analysis due to its moderate activity profile (PA14 $IC_{50}=(40\pm13)$ μM), availability, and overall ease of synthesis across the desired scaffolds for SAR consideration. The SAR for **4** was divided into six separate themes: deletion of the amide bond, linker chain modification, sliding of the amide bond, increased substitution of the amide bond, reversal of the amide bond directionality to mimic oroidin, and examination of a triazole isostere (Scheme 2).



Scheme 2. Core approaches to tuning the RA scaffold for antibiofilm activity.

Initially, it was planned to evaluate all of the target analogues against *Pseudomonas aeruginosa* PA14 biofilms. When active compounds were identified, IC_{50} values would then be determined. Additionally, the most active analogues in each subclass would then be tested for antibiofilm inhibition activity against *Acinetobacter baumannii* biofilms. Pending the identification of active compounds in these screens, *A. baumannii* IC_{50} values would be determined. Finally, the most active analogues would then be assayed for their ability to disperse established PA14 and/or *A. baumannii* biofilms.

Optimizing the aliphatic RA chain length: The first goal of the study was to further investigate the full extent of antibiofilm activity attainable by tuning the length of the aliphatic side chain. To this end, we synthesized compounds **10**–**12** as their HCl salts following our previously established chemistry[17] (Table 2).

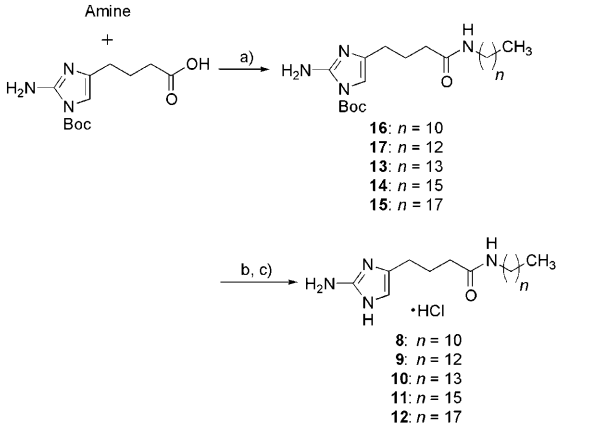
The hexadecyl- (**11**) and octadecyl-based (**12**) analogues displayed modest antibiofilm activity (PA14 IC_{50} values of 50 and 110 μM, respectively). Tetradecyl RA analogue **10** was slightly less active than **7** with a PA14 $IC_{50} = (2.9 \pm 1.2)$ μM, from which can be concluded that the highest activity for the RA scaffold should be a tridecyl RA analogue.

With that hypothesis, the tridecyl RA analogue **9** was synthesized and assayed for its ability to inhibit PA14 biofilm formation (Table 2). As anticipated, the tridecyl RA analogue **9** was a potent antibiofilm modulator with an IC_{50} value of (729 ± 85) nM against PA14. This makes **9** over threefold more active than **7** in reference to inhibition of PA14 biofilms and brings the activity profile of this class of compounds into the high nanomolar region. Analogue **8** was then synthesized to complete the aliphatic trend and corresponded well with the previously reported aliphatic data (PA14 $IC_{50} = (4.3 \pm 0.67)$ μM) as it was slightly less active than the dodecyl RA analogue **7**, but slightly more active than the decyl RA analogue **6**. This structure–activity relationship is graphically depicted in Figure 1. Growth curves were performed in the presence and absence of **9** with PA14 to ensure that the antibiofilm activity was not a result of a bactericidal effect. Bacterial-cell densities remained the same throughout a 24 h time period (Supporting Information).

These results show that by incrementally increasing the aliphatic chain length for the RA scaffold, we were able to identify one of the few nanomolar inhibitors of biofilm formation that have been disclosed.[19,34] It is also worth noting the correlation between the aliphatic chain length and the activity profile of the aliphatic RA analogues (Figure 1). Based upon this data, it would seem probable that these aliphatic RA analogues are eliciting their activity through binding a hydrophobic pocket. Surpassing the optimum chain length displays a rapid loss of activity as the analogues would loose their affinity for the binding pocket.

Lastly, it is important to point out that all of the identified aliphatic RA derivatives are still more active than the parent natural product oroidin.[16,17] Having now identified the most active aliphatic RA side chain and the break-point in activity, interest turned to how modifications of the core scaffold would tune antibiofilm activity with respect to the derivatization of the hexyl RA analogue **4**.

Table 2. Synthesis and antibiofilm activity of second-generation aliphatic RA analogues.[a]



Amine	Coupled product (yield [%])	Target (yield [%])	PA14 IC_{50} [μM]
tetradecylamine	**13** (24)	**10** (92)	2.9 ± 1.2
hexadecylamine	**14** (19)	**11** (92)	50
octadecylamine	**15** (13)	**12** (94)	110
undecylamine	**16** (31)	**8** (98)	4.3 ± 0.67
tridecylamine	**17** (25)	**9** (99)	0.73 ± 0.08

[a] Reaction conditions: a) EDC, HOBt, DMF; b) TFA, CH_2Cl_2; c) 2 M HCl/Et_2O.

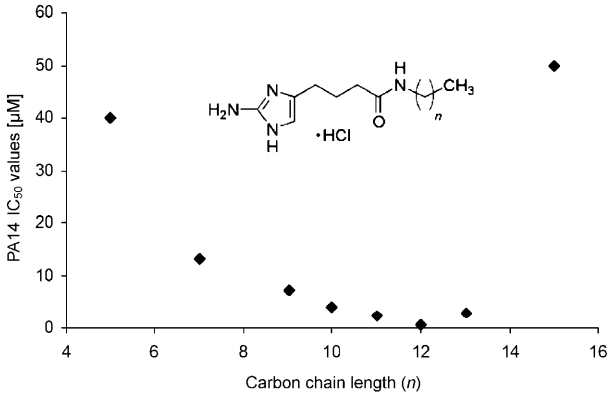


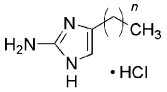
Figure 1. Correlation of aliphatic RA analogues and PA14 biofilm inhibition.

Deletion of the amide bond: From a synthetic standpoint, removal of the amide bond has a great advantage in simplifying the synthetic sequence for this class of compounds, most notably circumventing the low-yielding amide bond formation step. A quick search of commercially available acid chlorides revealed a large number of building blocks that, when derivatized, would be directly comparable to the RA scaffold. It also allowed for additional functionality, such as points of unsaturation along the chains, to be incorporated into the molecules. Ultimately, if these compounds were to show promise, additional analogue development could easily be executed through further manipulation of the double bond.[35,36]

Starting with a sampling of commercially available acid chlorides, we elected to synthesize several analogues that would mimic the aliphatic RA derivatives in overall length. Specifically, deletion of the amide bond in the hexyl RA analogue **4** would yield undecyl fatty acid analogue **19** (Scheme 3). Several unsaturated analogues were also prepared. The 2-AI targets were quickly accessed as outlined in Scheme 3. Briefly, the acid chlorides were homologated with diazomethane followed by quenching with concentrated HBr to afford the requisite α-bromoketones. The α-bromoketones were then condensed with Boc-guanidine in the presence of NaI followed by deprotection with acid and salt exchange to afford the target compounds.

Antibiofilm assessment of these analogues lacking the amide bond exhibited a range of potencies. The isopropyl-based fatty acid **25** exhibited modest activity, inhibiting biofilm formation 48% at 100 μM. The most active analogue obtained was the nonyl 2-AI fatty acid **18** with a PA14 IC_{50} value of (14±2.2) μM (Table 3). This was followed closely by the undecyl and tridecyl analogues (IC_{50} values of (19± 0.70) and (18±1.3) μM, respectively). With the success of the shorter-chain derivatives (**18**–**20**), longer analogues were analyzed (**21** and **22**), including several unsaturated derivatives (**23** and **24**), but these analogues were completely inactive toward inhibiting PA14 biofilms at 100 μM. Analogue **18** was chosen for PA14 growth-curve analysis to represent this class and in the presence or absence of **18**, PA14 cellular densities remained the same throughout a 24 h time period (Supporting Information).

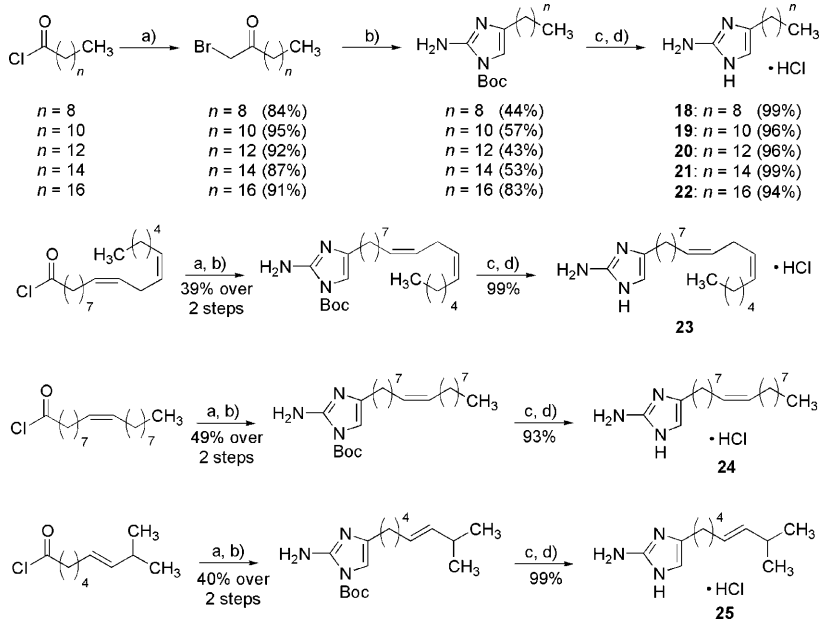
Table 3. Antibiofilm activity of an amide deletion subclass against PA14.



18-20

18 ($n=8$)	**19** ($n=10$)	**20** ($n=12$)
IC_{50}=(14±2.2) μM	IC_{50}=(18±0.70) μM	IC_{50}=(18±1.3) μM

In summary, removal of the amide bond from the aliphatic chain of the RA scaffold is not entirely detrimental to antibiofilm activity. The undecyl 2-AI fatty acid **19** is directly comparable to the hexyl RA derivative's overall chain length, thus showing that removal of the amide bond led to activity that was well over twofold more active than hexyl analogue **4**. Two additional derivatives were also shown to be potent inhibitors of PA14 biofilm formation. Although these compounds are significantly less active then tridecyl RA analogue **9**, simplification of the



Scheme 3. Deletion of the amide bond: a) i) CH_2N_2, Et_2O/CH_2Cl_2, 0 °C; ii) conc. HBr; b) Boc-guanidine, NaI, DMF; c) TFA, CH_2Cl_2; d) 2 M HCl/Et_2O. Boc: *tert*-butoxycarbonyl; TFA: trifluoroacetic acid.

synthetic approach allows rapid access to compounds of this class.

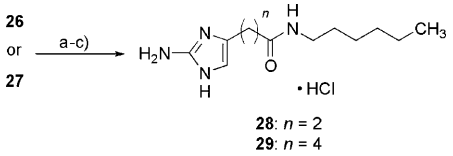
Linker modification: Examination of the majority of compounds comprising the oroidin class of natural products often reveals a three-carbon methylene linker between the 2-aminoimidazole (2-AI) head group and the pyrrole carboxamide group.[16] This three-carbon linker was previously confirmed to be required for maximum biological activity by a thorough oroidin template SAR in the context of *P. aeruginosa* antibiofilm activity.[16] Although this aspect was not detailed in our previous RA work (which was solely based on a three-carbon linker), we felt it necessary to revisit this structural modification and investigate the spacer effect in the RA class of molecules. Therefore, the two- and four-carbon Boc-2-AI carboxylic acid homologues necessary for amide bond coupling were synthesized (Scheme 4). Briefly, succinic anhydride was opened with benzyl alcohol followed by diazomethane/α-bromo homologation of the acid chloride to afford the α-bromo succinic benzyl ester. The α-bromoketone was then condensed with Boc-guanidine followed by hydrogenolysis of the benzyl ester to afford the two-carbon Boc-2-AI carboxylic acid **26**. Similarly, the four-carbon homologue was synthesized from the known monobenzyl ester of dipentanoic acid following the same procedure to deliver the intermediate acid **27** (Scheme 4).[37]

With these scaffolds in hand, coupling to hexylamine under carbodiimide conditions afforded derivatives comparable to the hexyl RA analogue **4** in that they were either a carbon atom shorter (**28**) or longer (**29**) in overall length from the 2-AI motif (Table 4). The two-methylene-unit analogue **28** had much lower activity than the parent three-carbon linker, displaying only 32% inhibition at 100 μM. Similarly, the four-methylene-unit linker **29** only inhibited PA14 biofilm formation 28% at 100 μM (Table 4).

Following the use of hexylamine as the coupling partner and lack of observed potencies with both linker homologues, we elected to investigate a few additional amines employing the two-carbon scaffold due to availability (Table 5). The two compounds prepared were chosen to probe whether a phenyl ring could mimic the activity of the aliphatic chains and recapitulate potency. It was anticipated that the *p*-bromo phenethyl RA analogue **30** would follow the same trend observed in other studies with increasing antibiofilm activity correlating to an increased degree of bromination.[14,16] Analogue **30** exhibited potent activity with an IC_{50} value of (15±3.9) μM against PA14, whereas the phenbutyl RA analogue **31** was less active with an IC_{50} value of (59±15) μM. Again, to demonstrate the nontoxic nature of these analogues on planktonic bacterial growth, growth-curve experiments with the *p*-bromo analogue **30** were performed. Both the treated and untreated cell densities remained identical during a 24 h time period (Supporting Information).

Ultimately either the addition or removal of a single methylene unit to the linker of the RA scaffold with the hexylamide drastically reduced antibiofilm potency. However, it was shown that activity could be regained with the addition of a phenyl ring. Compounds possessing increasing degrees of bromination similar to **30** along with other variously substituted ring systems are currently being formulated and evaluated for antibiofilm properties to further probe this observation.

Table 4. Synthesis and antibiofilm activity of modified linker analogues.[a]

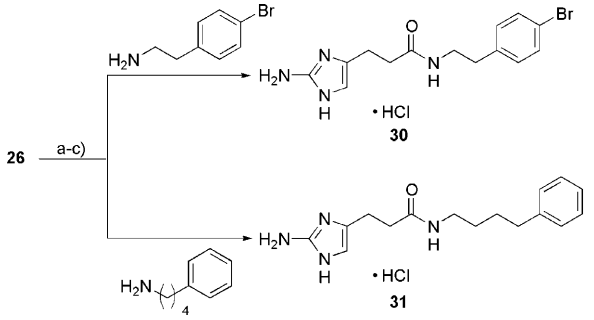


28: *n* = 2
29: *n* = 4

Target	Biofilm inhibition at 100 μM vs PA14 [%]
28	32±5
29	28±5

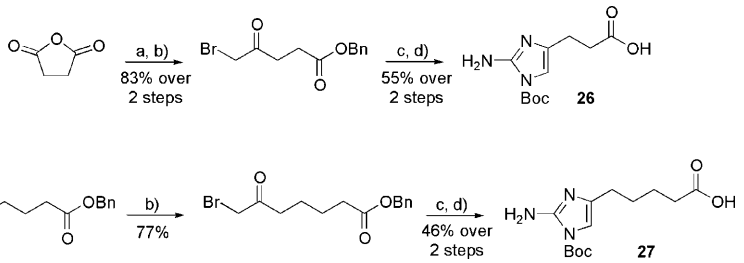
[a] Reaction conditions: a) hexylamine, EDC, HOBt, DMF; b) TFA, CH_2Cl_2; c) 2 M HCl/Et_2O.

Table 5. Synthesis and antibiofilm activity of phenylamine analogues.[a]



Target	PA14 IC_{50} [μM]
30	15±3.9
31	59±15

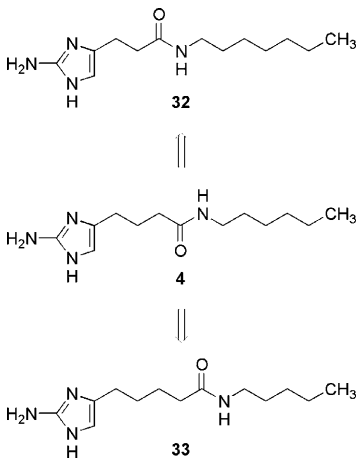
[a] Reaction conditions: a) EDC, HOBt, DMF; b) TFA, CH_2Cl_2; c) 2 M HCl/Et_2O.



Scheme 4. Synthesis of Boc-2-AI linker acids: a) BnOH, TEA, DMAP, CH_2Cl_2 (87%); b) i) $(COCl)_2$, DMF (cat.), CH_2Cl_2; ii) CH_2N_2, Et_2O/CH_2Cl_2, 0 °C; iii) conc. HBr; c) Boc-guanidine, DMF; d) H_2 (1 atm), 10% Pd/C, THF. DMAP: 4-dimethylaminopyridine; TEA: triethylamine.

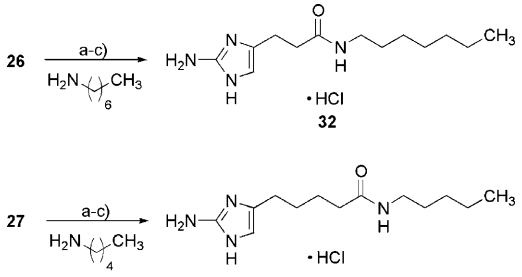
Sliding the amide bond: We envisioned using the hexyl RA analogue as our starting point

and sliding the amide bond one carbon atom in each direction along the chain (Scheme 5). This would be complementary to the linker-chain modification study detailed above. By using the aforementioned two- and four-carbon homologue carboxylic acids, **26** was coupled to heptylamine whereas **27** was coupled to pentylamine to afford the two analogues both with identical total chain lengths in reference to the parent hexyl RA analogue **4** (Table 6). Evaluation of these two analogues for biofilm inhibitory activity against PA14 showed that the two-carbon analogue **32** (PA14 $IC_{50} = (43 \pm 8)$ μM) was only slightly less active than the parent three-carbon hexyl RA **4**, but this difference was negligible due to associated error (Table 6). The four-carbon analogue **33** was significantly less active, only being able to inhibit PA14 biofilm formation 24% at 100 μM.



Scheme 5. Sliding the amide bond.

Table 6. Synthesis and antibiofilm activity of slider analogues.[a]

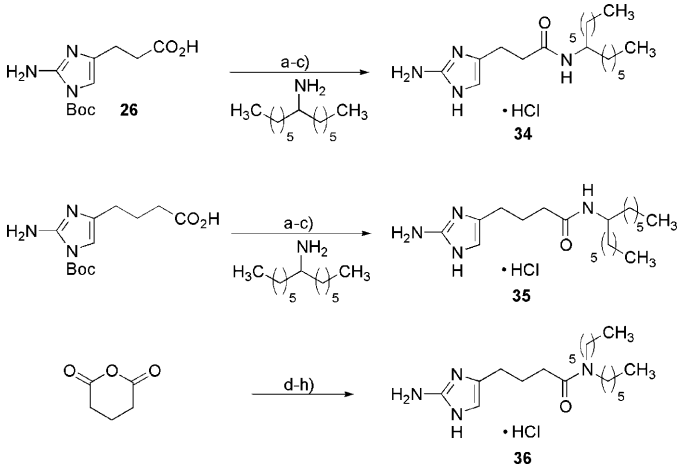


Target	Biofilm inhibition at 100 μM vs PA14 [%]	PA14 IC_{50} [μM]
32	74 ± 2	43 ± 8
33	24 ± 5	n.d.

[a] Reaction conditions: a) EDC, HOBt, DMF; b) TFA, CH_2Cl_2; c) 2 M HCl/Et_2O. n.d. = not determined.

Increased substitution: Long aliphatic amides on a RA 2-AI scaffold afforded compounds that were extremely active at inhibiting PA14 biofilm formation.[17] Increased molecular diversity can be accessed through installation of an additional aliphatic chain to the amide moiety in anticipation that it would be able to further enhance activity.

Therefore, both a secondary and tertiary dihexyl RA derivative were synthesized to simultaneously test for the affect of additional aliphatic chains and the necessity of the NH amide proton for antibiofilm activity. The synthesis of the secondary dihexyl derivatives are outlined in Scheme 6.

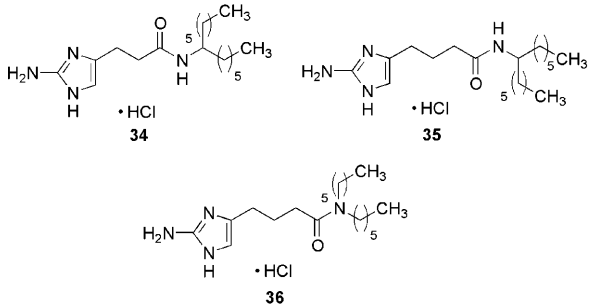


Scheme 6. Synthesis of additionally substituted analogues: a) EDC, HOBt, DMF; b) TFA, CH_2Cl_2; c) 2 M HCl/Et_2O; d) dihexylamine, TEA, DMAP, CH_2Cl_2 (87%); e) i) $(COCl)_2$, DMF (cat.), CH_2Cl_2; ii) CH_2N_2, Et_2O/CH_2Cl_2, 0 °C; iii) conc. HBr; f) Boc-guanidine, DMF (27% over 2 steps); g) TFA, CH_2Cl_2; h) 2 M HCl/Et_2O (94%). EDC: *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide; HOBt: 1-hydroxybenzotriazole.

Coupling the three- and two-carbon carboxylic acids with the known branched secondary hexaheptylamine[38] under EDC conditions followed by deprotection and salt exchange afforded the target secondary dihexyl RA analogues **34** and **35**. Synthesis of the tertiary dihexyl RA analogue **36** was executed through opening of glutaric anhydride with dihexylamine and transforming the resulting acid intermediate into the final 2-AI target.

Upon biological evaluation of these analogues, the tertiary derivative **36** displayed moderate inhibitory activity against PA14 biofilms with an IC_{50} value of (60 ± 12) μM (Table 7). It is notable that within the experimental errors

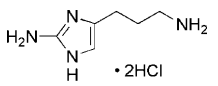
Table 7. Antibiofilm activity of additionally substituted RA analogues.



PA14 IC_{50} [μM]		
34: 18 ± 1.3	**35**: 16 ± 1.8	**36**: 60 ± 12

reported, this activity is almost identical to the monohexyl RA analogue **4**. Evaluation of the three-carbon secondary dihexyl RA analogue **35** against inhibiting PA14 biofilms, however, gave an IC_{50} value of (16 ± 1.8) μM that was over twofold more active than the monohexyl RA analogue. The two-carbon secondary dihexyl RA analogue **34** was also active at inhibiting PA14 biofilms with an $IC_{50} = (18 \pm 1.3)$ μM, essentially identical to the three-carbon homologue. Growth curve experiments with compound **35** validated that cellular densities remained identical over a 24 h time period (Supporting Information). Clearly, activity increases with the increased substitution alpha to the amide, and activity is still retained when the NH amide proton is replaced with an aliphatic chain in this subset of compounds. Furthermore, it is anticipated that future analogues, such as those with longer disubstituted aliphatic chains (i.e., di-tridecyl), will also display increased potency in comparison to those containing just a single aliphatic chain.

A native amide approach: Both amide bond deletion and additional substitution of the amide functionality were efforts to elucidate if the aforementioned region of the molecule was critical to eliciting antibiofilm activity. Some modifications in structure are well tolerated within the context of antibiofilm activity. Throughout the synthetic efforts on this project, one aspect that we had not addressed was the construction of dihydrooroidin analogues with aliphatic side chains that contained the amide in the native orientation. Evaluation of these derivatives is complicated by the lack of synthetic methods to rapidly acylate a core 2-AI derivative to generate molecular diversity. Typical methods to acylate core 2-AI derivatives, such as **37**, rely on the use of trichloroesters as coupling partners.[21] The use of 4-(3-aminopropyl)-2-aminoimidazole **37** in acylation reactions involving the oroidin class of molecules leaves much to be desired in terms of yields and ease of purification.[16] Upon close examination of the literature, a new approach was developed to circumvent these problems while also again taking advantage of the ability to synthesize a large number of chemically unique derivatives. The idea was to develop acylation chemistry around a masked amine relative of our established Boc-2-AI carboxylic acid intermediates (i.e., **26** and **27**).



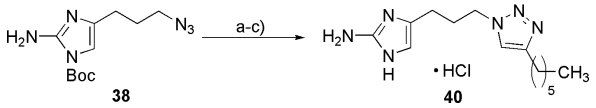
37

Advancement of the known[39] 4-azido-butyric acid proceeded smoothly to afford the Boc-2-AI azide **38** (Scheme 7). This intermediate was reduced with Pd/C under a hydrogen atmosphere followed by in situ acylation employing heptanoic anhydride to afford the native amide analogue **39** in a high yield over two steps (70%). This pathway also allows access to a Boc-protected derivative of **37**, which is not accessible through the established routes to this intermediate. Deprotection afforded **39**, the native amide hexyl analogue of **4**, thus producing a compound that contains the amide directionality seen in the natural products.

Assays performed by investigating this analogue against PA14 proved very interesting as it was completely inactive within the concentrations tested, yielding $< 5\%$ biofilm inhibition at 100 μM. This result is in stark contrast to the activity reported for **4** and is very instructive in understanding the impact of amide directionality coupled with an appropriate aliphatic chain on antibiofilm properties. Further development and tuning of this methodology is currently under investigation to increase chemical diversity and antibiofilm properties around this portion of the molecule.

Examination of a triazole isostere: En route to synthesizing the natural amide hexyl analogue, it was also observed that construction of a triazole isostere was possible through the utilization of the pendant azide functionality.[40] The overall dipolar moment of a triazole system is larger than that of an amide bond, thus making its hydrogen-bonding donor and acceptor properties more pronounced.[41,42] Due to the recent success of a small library of 2-AI/triazole conjugates in inhibiting and dispersing various medically relevant biofilms,[19] an analogue of this nature would be a valuable addition to the current SAR study. To this end, the Boc-2-AI azide **38** was reacted with 1-octyne in a [3+2] copper-catalyzed click reaction followed by deprotection to smoothly afford the triazole hexyl isostere **40** (Table 8). The triazole analogue **40** was observed to possess an IC_{50} value of (27 ± 4) μM, allowing for retainment of activity in comparison with **4**. A PA14 growth curve was also performed in the presence and absence of **40**. Over a 24 h time period, both the treated and untreated cell densities remained identical (Supporting Information). Additional libraries taking advantage of both the speed and diversity of the

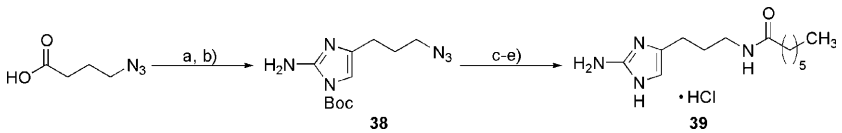
Table 8. Synthesis and antibiofilm activity of a triazole isostere.[a]



38 **40**

Target	PA14 IC_{50} [μM]
40	27 ± 4

[a] Reaction conditions: a) 1-octyne, $CuSO_4 \cdot 5H_2O$ (20 mol%), Na Ascorbate (10 mol%), H_2O/EtOH 1:1 (55%); b) TFA, CH_2Cl_2; c) 2 M HCl/Et_2O (99%).

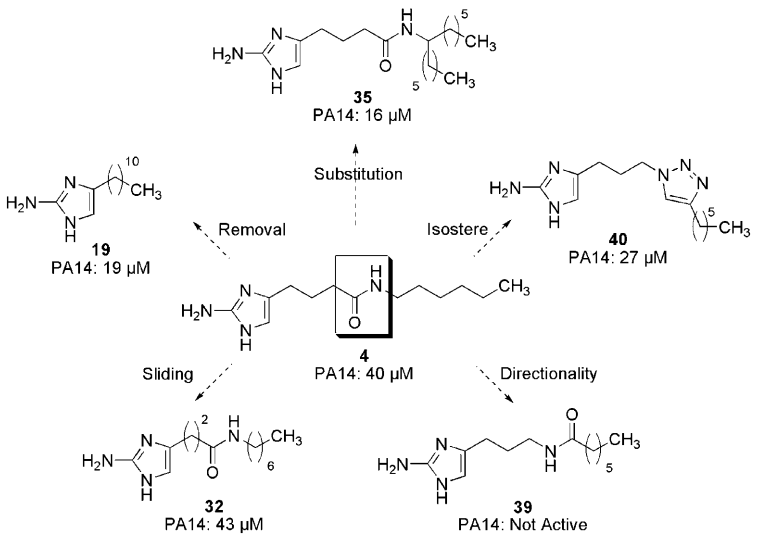


38 **39**

Scheme 7. A native amide bond approach: a) i) $(COCl)_2$, DMF (cat.), CH_2Cl_2; ii) CH_2N_2, Et_2O/CH_2Cl_2, 0 °C; iii) conc. HBr (90%); b) Boc-guanidine, DMF (63%); c) i) H_2 (1 atm), 10% Pd/C, THF; ii) heptanoic anhydride (70%); d) TFA, CH_2Cl_2; e) 2 M HCl/Et_2O (99%).

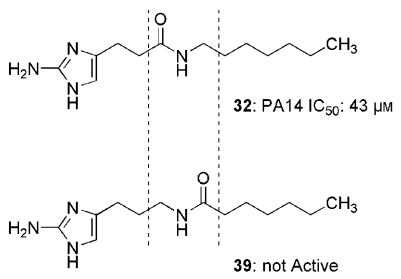
click reaction with the newly developed scaffold **38** are currently being generated.

PA14 biofilm inhibition: Activity profile comparisons: A summary of the results obtained via amide bond modifications is depicted in Scheme 8. The most revealing observation about the current RA SAR study (Scheme 8) was the lack of activity exhibited by the native amide hexyl congener **39** relative to the RA derivative **4**. The native amide **39** was completely inactive, and unable to inhibit PA14 biofilm formation even at the highest concentration tested (100 μM). This result confirms that the directionality of the amide bond can have a significant impact on antibiofilm activities. Interestingly, **32** (which bears the amide carbonyl towards the side of the 2-AI motif) was an active biofilm modulator, in stark contrast to **39** (Scheme 9).



Scheme 8. PA14 inhibition IC_{50} values from selected SAR analogues.

Additionally, exchange of the amide bond with a triazole surrogate to produce **40** showed that activity could be slightly enhanced with this substitution. These observations again, may be hinting towards a crucial placement of the amide carbonyl/triazole moiety to elicit a maximum biological effect through possible intramolecular or intermolecular interactions. Both the two- (**32**) and four-carbon (**33**) analogues were less active than the parent three-carbon hexyl RA analogue.



Scheme 9. Amide orientation comparison.

While sliding the amide bond and changing the chain length tended to reduce activity, increasing the substitution adjacent to the amide bond tended to cause an increase in activity. Synthesis of the secondary dihexyl derivative **35** increased the PA14 biofilm inhibition activity twofold. While this may be attributable to an increase in the longest chain (7 C atoms for **35**, 6 C atoms for **4**), the activity corresponds closely to that of the octyl RA analogue, which indicates that the increased substitution is modulating the activity.[17] The tertiary-amide derivative **36** is only slightly less active then the monohexyl RA analogue.

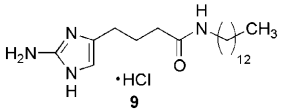
Deletion of the amide bond provided several active fatty acid derived 2-AI analogues (i.e., **19**), which displayed increased activity in comparison to their RA relatives. There was, however, a clear cut-off point for the potency of this class of molecules as only the shorter-chain saturated derivatives were active.

***Acinetobacter baumannii* biological evaluation**: To this point, we have exclusively employed PA14 to evaluate the current library as antibiofilm agents. To determine the broad spectrum of activity of the compounds, it was deemed necessary to assay our most active and structurally diverse analogues against the Gram-negative γ-proteobacterium *Acinetobacter baumannii*.

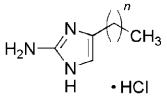
Nine of the most active analogues obtained from the *Pseudomonas* biofilm inhibition assays underwent a preliminary screen for biofilm inhibition activity against *A. baumannii* (Table 9). Three of these derivatives (**30**, **36**, and **40**) showed only marginal activity at 100 μM, which was opposed to the potency observed for these analogues against PA14. Tridecyl RA analogue **9**, the most active compound with activity in the high nanomolar range against PA14, was also noticeably less potent against *A. baumannii* ($IC_{50} = (26 \pm 3)$ μM). The analogues derived from the subclass of compounds in which the amide bond had been removed (**18**–**20**) displayed the greatest activity out of any of the compounds assayed against *Acinetobacter*. It was observed that as the chain length increased, potency increased culminating in the most potent derivative **20** ($IC_{50} = (13 \pm 0.70)$ μM). This trend is opposite to that observed for PA14 inhibition activity, as the shortest-chain fatty acid derivative **18** was the most potent.

Growth curves were then performed with several of the active compounds (**9**, **19**, **20**, and **35**) at their respective IC_{50}

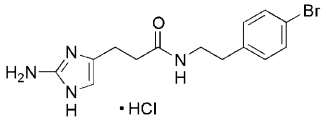
Table 9. Antibiofilm activity against *A. baumannii*.



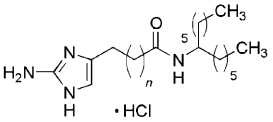
9



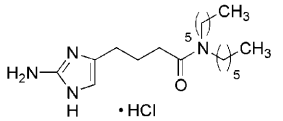
18: $n = 8$
19: $n = 10$
20: $n = 12$



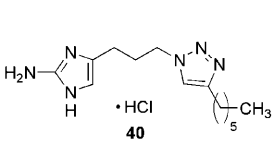
30



34: $n = 1$
35: $n = 2$



36



40

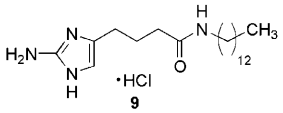
Analogue	Biofilm inhibition at 100 μM vs *A. baumannii* [%]	*A. baumannii* IC_{50} [μM]
9	≥95	26±3
18	≥95	43±1
19	≥95	13±0.70
20	≥95	8.0±0.32
30	18±3	n.d.
34	≥95	51±1
35	≥95	38±1
36	34±4	n.d.
40	22±4	n.d

values. Surprisingly, the compounds seemed to be eliciting some of their antibiofilm properties through a bacteriostatic effect, as evidenced by the lack of bacterial growth for the bacteria that were dosed with compound. *A. baumannii* growth was suppressed by the analogues for at least 9 h, but by the 24 h time-point, the treated cell densities were nearly the same as the untreated cell densities. This led to growth curve experiments performed at the analogues respective IC_{30} values (**9**: 15, **19**: 10, **20**: 6.3, **35**: 37 μM) as a means to determine the toxicity of these compounds at lower concentrations. At the IC_{30} value, growth was evident after only 3 h, but was still slower relative to untreated samples. After 24 h at the IC_{30}, the cell densities were, again, relatively identical to the untreated cell densities. This appears to indicate that these compounds have a very small window between inhibiting biofilm formation and inhibiting planktonic *A. baumannii* growth. Overall, these compounds are still eliciting a biofilm inhibitory effect that is unique to this class of compounds. Their *A. baumannii* biofilm inhibitory activity may be modulated by slight bacteriostatic effects, but during the course of the assay (24 h), the treated bacteria have enough time to become homeostatic with respect to the untreated controls as evidenced by the growth curve.

Despite the overall decrease in the general ability to inhibit *Acinetobacter* biofilm formation relative to PA14, the inhibition data presented is still noteworthy. Although the *A. baumannii* antibiofilm-modulating properties of the RA class of compounds may be due, in part, to bacteriostatic effects, their activity lends hope to the RA library's ability to possibly inhibit other biofilms formed by medically relevant bacteria, thus solidifying their importance as novel small molecules in the antibiofilm arena.

Dispersion of established biofilms: Perhaps a more important characteristic of small molecules known to inhibit biofilm formation is their ability to disperse pre-existing biofilms (Table 10). This is significant from a biomedical stand-

Table 10. Selected compounds with biofilm dispersing properties.



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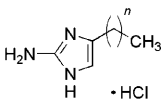
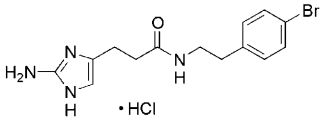
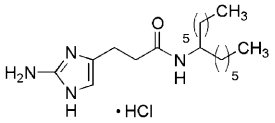


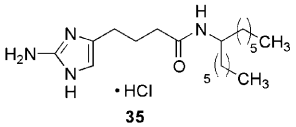
18: $n = 8$
19: $n = 10$
20: $n = 12$



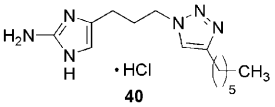
30



34



35



40

Analogue	*P. aeruginosa*: PA14 dispersion 100 μM [%]	EC_{50} [μM]	*A. baumannii* dispersion 100 μM [%]	EC_{50} [μM]
9	73±2	54±6	38±1	75±13
18	≥95	64±3	39±3	131±10
19	≥95	67±5	72±1	68±2
20	24±4	n.d.	37±3	121±9
30	18±3	n.d.	<5	n.d.
34	n.d.	n.d.	77±1	60±2
35	≥95	26±2	65±3	61±3
40	33±6	n.d	n.d.	n.d.

point as treatment for infections usually begins after biofilm formation has been initiated and current therapies may be of no use. It was shown previously that our most active RA derivative **7** (obtained from the first-generation library) acted as both a superior inhibitor and dispersal agent against *Pseudomonas* PAO1 and PA14 biofilms. With the discovery of more potent biofilm inhibitors, it was investigated as to whether this success would also translate into enhanced biofilm dispersal activity. To test this, PA14 and *A. baumannii* biofilms were first allowed to form in the absence of compound before being dosed with our most active derivatives that encompassed various structural characteristics and potencies against both strains of bacteria.

The stringency applied to the dispersion screens was similar to that used in the inhibition assays. Dispersal activity was first assessed at 100 μM to determine which compounds would then be moved on further for EC_{50} value determina-

tion. One general trend observed for dispersion activity was that all biofilm dispersion EC_{50} values were higher than the respective compounds biofilm inhibition IC_{50} values regardless of the bacterial strain employed.

The hope of duplicating the dispersal activity of **7** with the newly identified most active PA14 biofilm inhibitor **9** was not realized. This compound only showed an average dispersal activity against PA14 with an EC_{50} value of (54 ± 6) μM. The only other compound of exceptional note was the secondary dihexyl derivative **35**, the EC_{50} dispersal activity of which is, within error, just as potent as **7**. Two of the shorter-chain fatty acid analogues **18** and **19** also showed moderate biofilm dispersing properties, whereas the triazole analogue **40**, which was a potent PA14 biofilm inhibitor, did not posses the same dispersal activity.

The most active small molecules identified in the dispersal of existing *Acinetobacter baumannii* biofilms were those bearing the dihexyl side chains on the two- (**34**) or three-carbon (**35**) amide bond linker (EC_{50} values of (60 ± 2) and (61 ± 3) μM, respectively). Closely following in potency was the fatty acid derivative **19** and the tridecyl reverse amide analogue **9**.

Conclusions

Overall, the most insightful information obtained from this SAR study involved the activities of the analogues bearing the native oroidin amide directionality and its replacement with a triazole isostere. Intriguingly, no antibiofilm activity was observed for the natural amide congener **39**, which is in contrast to the parent hexyl RA **4**. Also, a direct amide bond directionality comparison can be made with analogues **32** and **39** (Scheme 9). Analogue **32** was shown to be nearly as active as the hexyl RA **4**, whereas **39**, with the native amide orientation, displayed no activity. Additionally, the triazole isostere **40** recapitulated the activity of RA **4**, which will allow for further SAR analysis.

It remains to be seen whether other derivatives bearing the amide orientation present in the natural products have the ability to be as potent as those within the RA scaffold. Exploitation of the click reaction and reductive acylation methodology outlined above will ultimately allow this point to be addressed head-on and is currently under intensive investigation.

Further SAR information indicates that increased substitution directly adjacent to the amide bond is an effective means of increasing activity. Applying this to the newly identified tridecyl RA analogue **9**, it may be possible to further enhance its activity and efforts to address this issue are also being formulated.

Additionally, removal of the amide bond entirely allowed for the facile multigram generation of several fatty acid 2-AI derivatives (**18**–**20**) that were shown to possess antibiofilm activity. However, the range of active analogues does not mirror that of the RA aliphatic chain subclass, but the three active fatty acid analogues identified were active antibiofilm modulators. The fatty acid 2-AI analogues were also shown to possess some bacteriostatic properties during the early stages of *A. baumannii* biofilm development.

Despite the inability of our most active PA14 biofilm inhibitor (tridecyl RA analogue **9**) to become the most potent PA14 biofilm dispersal agent, the dispersal activity of selected members from the library is noteworthy due to the potency of analogue **35** in being able to disperse both PA14 and *A. baumannii* biofilms. It is also interesting that a few analogues that were active in the inhibition assays lacked significant dispersal activity (i.e., compounds **30** and **40**). This trend is not without precedence as other active antibiofilm molecules have demonstrated similar activity profiles against various bacterial biofilms.[14,18,34]

In conclusion, we have demonstrated that the RA class of 2-aminoimidazoles has provided a fertile avenue for the exploration and development of numerous biofilm modulators. Information obtained with the SAR profile from this class of compounds should allow for further analogue tuning and ultimately facilitate the construction of even more potent 2-AI antibiofilm derivatives. With the lack of new classes of antibiotics and increased multidrug resistance, the need for alternative approaches to mitigate infectious diseases is sorely needed.

Experimental Section

General: Stock solutions (100, 50, 10, 1 mM) of all compounds assayed for biological activity were prepared in DMSO and stored at room temperature. The amount of DMSO used in both inhibition and dispersion screens did not exceed 1% (by volume). *P. aeruginosa* PA14 was graciously supplied by the Wozniak group at Wake Forest University School of Medicine and by the O'Toole Group at Dartmouth Medical School. *A. baumannii* was purchased from ATCC (ATCC 19606).

General static inhibition assay protocol for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: An overnight culture of the wild-type strain was subcultured at an OD_{600} of 0.01 into LBNS (PA14) or LB (*A. baumannii*) along with a predetermined concentration of the small molecule to be tested for biofilm inhibition. Samples were then aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. The microtiter dishes were covered and sealed before incubation under stationary conditions at 37°C for 24 h. After that time, the medium was discarded and the plates thoroughly washed with water. The wells were then inoculated with a 0.1% aqueous solution of crystal violet (100 μL) and allowed to stand at ambient temperature for 30 min. Following another thorough washing with water, the remaining stain was solubilized with 200 μL of 95% ethanol. Biofilm inhibition was quantitated by measuring the OD_{540} for each well by transferring 125 μL of the ethanol solution into a fresh polystyrene microtiter dish for analysis.

General static dispersion assay protocols for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: An overnight culture of the wild-type strain was subcultured at an OD_{600} of 0.05 into LBNS (PA14) or LB (*A. baumannii*) and then aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. The microtiter dishes were covered and sealed before incubation under stationary conditions at room temperature to allow formation of the biofilms. After 24 h, the medium was discarded and the plates thoroughly washed with water. Fresh medium containing the appropriate concentration of compound was then added to the wells. The plates were again sealed and this time incubated under stationary conditions at 37°C. After 24 h, the media was discarded from the wells and the plates washed thoroughly with water. The wells were inoculated with a

0.1% aqueous solution of crystal violet (100 μL) and allowed to stand at ambient temperature for 30 min. Following another thorough washing with water, the remaining stain was solubilized with 200 μL of 95% ethanol. Biofilm dispersion was quantitated by measuring the OD_{540} for each well by transferring 125 μL of the ethanol solution into a fresh polystyrene microtiter dish for analysis. Percent dispersion was calculated by comparison of the OD_{540} for established biofilm (untreated) versus treated established biofilm under identical conditions.

Chemistry: All reagents including anhydrous solvents used for the chemical synthesis of the library were purchased from commercially available sources and were used without further purification unless otherwise noted. All reactions were run under either a nitrogen or argon atmosphere. Flash silica-gel chromatography was performed with 60 Å mesh standard grade silica gel from Sorbtech. ^{1}H and ^{13}C NMR spectra were obtained by using Varian 300 or 400 MHz spectrometers. NMR solvents were purchased from Cambridge Isotope Labs and used as is. Chemical shifts are given in parts per million relative to [D$_6$]DMSO ($\delta = 2.50$ ppm), CD_3OD ($\delta = 3.34$ ppm), and $CDCl_3$ ($\delta = 7.27$ ppm) for proton spectra and relative to [D$_6$]DMSO ($\delta = 39.51$ ppm), CD_3OD ($\delta = 49.86$ ppm), and $CDCl_3$ ($\delta = 77.21$ ppm) for carbon spectra with an internal TMS standard. HRMS were obtained at the North Carolina State Mass Spectrometry Laboratory for Biotechnology. ESI experiments were carried out on an Agilent LC-TOF mass spectrometer.

Optimizing the RA chain length: General EDC/HOBt procedure: 2-Amino-4-(3-carboxy-propyl)imidazole-1-carboxylic acid *tert*-butyl ester[17] (0.100 g, 0.371 mmol), HOBt (0.100 g, 0.742 mmol), EDC (0.142 g, 0.742 mmol), and diisopropylethylamine (0.260 mL, 1.48 mmol) were dissolved in anhydrous DMF (3 mL) and allowed to stir for 15 min. The appropriate amine coupling partner (0.780 mmol) was then added and the solution was stirred at ambient temperature until completion was evident by TLC analysis. The reaction was concentrated under reduced pressure and the residue partitioned between ethyl acetate (20 mL) and water (10 mL). The organic layer was successively washed with water (3× 10 mL), 1 M HCl (3×10 mL), sat. $NaHCO_3$ (2×10 mL), and brine (10 mL) before being dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by flash column chromatography (2–10% MeOH/CH_2Cl_2) to afford the target compounds.

2-Amino-4-(3-tetradecylcarbamoyl-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (13): By using the general procedure, the title compound **13** (0.042 g, 24%) was formed as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO):$\delta = 7.72$ (m, 1H), 6.50 (s, 1H), 6.37 (s, 2H), 3.00 (q, 2H, $J =$ 6.4 Hz), 2.21 (t, 2H, $J = 6.8$ Hz), 2.04 (t, 2H, $J = 7.6$ Hz), 1.71 (m, 2H), 1.53 (s, 9H), 1.36 (m, 2H), 1.23 (brs, 22H), 0.85 ppm (t, 3H, $J = 5.2$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 171.64$, 149.91, 148.92, 138.26, 105.79, 84.03, 38.32, 34.90, 31.32, 29.16, 29.07, 29.04, 28.74, 28.02, 27.87, 27.51, 27.18, 26.41, 24.07, 22.12, 13.96; HRMS (ESI): m/z: calcd for $C_{26}H_{48}N_4O_3$: 464.3726 $[M]^+$; found: 464.3742.

2-Amino-4-(3-hexadecylcarbamoyl-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (14): By using the general procedure, the title compound **14** (0.034 g, 19%) was formed as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.72$ (m, 1H), 6.50 (s, 1H), 6.39 (s, 2H), 3.00 (q, 2H, $J =$ 6.4 Hz), 2.21 (t, 2H, $J = 7.2$ Hz), 2.04 (t, 2H, $J = 7.2$ Hz), 1.71 (m, 2H), 1.53 (s, 9H), 1.36 (m, 2H), 1.23 (brs, 26H), 0.85 ppm (t, 3H, $J = 5.2$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 171.63$, 149.89, 148.93, 138.33, 105.79, 84.01, 38.30, 34.89, 31.30, 29.14, 29.03, 28.71, 27.51, 27.19, 26.38, 24.06, 22.10, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{28}H_{52}N_4O_3$: 492.4039 $[M]^+$; found: 492.4033.

2-Amino-4-(3-octadecylcarbamoyl-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (15): By using the general procedure, the title compound **15** (0.025 g, 13%) was formed as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.73$ (m, 1H), 6.50 (s, 1H), 6.38 (s, 2H), 3.00 (q, 2H, $J =$ 6.4 Hz), 2.21 (t, 2H, $J = 7.2$ Hz), 2.03 (t, 2H, $J = 7.2$ Hz), 1.70 (m, 2H), 1.53 (s, 9H), 1.35 (m, 2H), 1.22 (brs, 30H), 0.85 ppm (t, 3H, $J = 6.0$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 171.61$, 149.84, 148.90, 138.38, 105.76, 83.99, 38.28, 24.88, 31.24, 29.10, 28.96, 28.64, 27.85, 27.48, 27.18, 26.33, 24.04, 22.04, 13.90 ppm; HRMS (ESI): m/z: calcd for $C_{30}H_{56}N_4O_3$: 520.4352 $[M]^+$; found: 520.4355.

2-Amino-4-(3-undecylcarbamoyl-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (16): By using the general procedure, 2-amino-4-(3-carboxy-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.150 g, 0.58 mmol) gave the title compound **16** (0.073 g, 31%) as a tan-colored solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 7.72$ (t, 1H, $J = 7.2$ Hz), 6.50 (s, 1H), 6.37 (s, 2H), 3.00 (q, 2H, $J = 6.9$ Hz), 2.21 (t, 2H, $J = 6.9$ Hz), 2.04 (t, 2H, $J = 7.2$ Hz), 1.70 (quint., 2H, $J = 7.5$ Hz), 1.53 (s, 9H), 1.36 (m, 2H), 1.23 (brs, 16H), 0.85 ppm (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 171.69$, 149.90, 148.93, 138.38, 105.81, 84.05, 38.33, 34.92, 31.30, 29.15, 29.01, 28.34, 27.52, 27.21, 26.39, 24.09, 22.11, 13.97 ppm; HRMS (ESI): m/z: calcd for $C_{23}H_{42}N_4O_3$: 422.3257 $[M]^+$; found: 422.3257.

2-Amino-4-(3-tridecylcarbamoylpropyl)imidazole-1-carboxylic acid *tert*-butyl ester (17): By using the general procedure, 2-amino-4-(3-carboxy-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.150 g, 0.580 mmol) gave the title compound **17** (0.062 g, 25%) as a tan-colored solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO). $\delta = 7.72$ (t, 1H, $J = 7.2$ Hz), 6.50 (s, 1H), 6.37 (s, 2H), 3.00 (q, 2H, $J = 6.9$ Hz), 2.21 (t, 2H, $J = 6.9$ Hz), 2.04 (t, 2H, $J = 7.2$ Hz), 1.70 (quint., 2H, $J = 7.5$ Hz), 1.53 (s, 9H), 1.36 (m, 2H), 1.23 (brs, 20H), 0.85 ppm (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 171.70$, 149.92, 148.96, 138.39, 105.81, 84.05, 38.38, 34.92, 31.32, 29.16, 29.03, 28.73, 27.53, 27.22, 26.40, 24.09, 22.12, 13.97 ppm; HRMS (ESI): m/z: calcd for $C_{25}H_{46}N_4O_3$: 450.3567 $[M]^+$; found: 450.3576.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-undecyl-butyramide hydrochloride (8): A solution of **16** (0.027 g, 0.064 mmol) in anhydrous dichloromethane (1 mL) was cooled to 0°C. TFA (1.0 mL) was charged into the flask and the reaction was stirred for 6 h. After this time, the reaction was evaporated to dryness and toluene (2 mL) was added. Again the mixture was concentrated and the process repeated. The resulting TFA salt was dissolved in dichloromethane (1 mL), and 2 M HCl in diethyl ether (0.5 mL) was added followed by cold diethyl ether (8 mL). The precipitate was collected by filtration and washed with diethyl ether (3 mL) to yield the target compound **8** (0.023 g, 99%) as a white amorphous solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 12.09$ (s, 1H), 11.65 (s, 1H), 7.85 (m, 1H), 7.30 (s, 2H), 6.55 (s, 1H), 3.00 (q, 2H, $J = 5.7$ Hz), 2.38 (t, 2H, $J =$ 7.5 Hz), 2.07 (t, 2H, $J = 7.5$ Hz), 1.73 (quint., 2H, $J = 7.5$ Hz), 1.36 (m, 2H), 1.23 (brs, 16H), 0.85 ppm (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 171.29$, 146.74, 126.34, 108.64, 38.40, 34.38, 31.27, 29.12, 29.01, 28.98, 28.72, 28.68, 26.41, 23.86, 23.60, 22.07, 13.93 ppm; HRMS (ESI): m/z: calcd for $C_{18}H_{34}N_4O$: 322.2733 $[M]^+$; found: 322.2744.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-tridecyl-butyramide hydrochloride (9): By using the same general procedure as that used for the synthesis of **8**, **17** (0.028 g, 0.062 mmol) gave the title compound **9** (0.024 g, 99%) as a white amorphous solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 12.08$ (s, 1H), 11.66 (s, 1H), 7.85 (m, 1H), 7.30 (s, 2H), 6.55 (s, 1H), 3.00 (q, 2H, $J = 5.7$ Hz), 2.38 (t, 2H, $J = 7.5$ Hz), 2.07 (t, 2H, $J = 7.5$ Hz), 1.73 (quint., 2H, $J = 7.5$ Hz), 1.36 (m, 2H), 1.23 (brs, 20H), 0.85 ppm (t, 3H, $J =$ 6.9 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 171.29$, 146.74, 126.34, 108.64, 38.40, 34.38, 31.27, 29.12, 29.01, 28.98, 28.72, 28.68, 26.41, 23.86, 23.60, 22.07, 13.93 ppm; HRMS (ESI): m/z: calcd for $C_{20}H_{38}N_4O$: 350.3046 $[M]^+$; found: 350.3052.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-tetradecyl-butyramide hydrochloride (10): By using the same general procedure as that used for the synthesis of **8**, **13** (0.030 g, 0.064 mmol) gave the title compound **10** (0.024 g, 92%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 12.05$ (s, 1H), 11.62 (s, 1H), 7.83 (m, 1H), 7.31 (s, 2H), 6.55 (s, 1H), 3.00 (q, 2H, $J =$ 6.4 Hz), 2.37 (t, 2H, $J = 7.2$ Hz), 2.07 (t, 2H, $J = 7.2$ Hz), 1.73 (m, 2H), 1.35 (m, 2H), 1.23 (brs, 22H), 0.85 ppm (t, 3H, $J = 6.4$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 171.30$, 146.72, 126.38, 108.67, 38.41, 34.39, 31.28, 29.13, 29.03, 28.99, 28.73, 28.69, 26.42, 23.86, 23.61, 22.08, 13.94 ppm; HRMS (ESI): m/z: calcd for $C_{21}H_{40}N_4O$: 364.3203 $[M]^+$; found: 364.3197.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-hexadecyl-butyramide hydrochloride (11): By using the same general procedure as that used for the synthesis of **8**, **14** (0.029 g, 0.059 mmol) gave the title compound **11** (0.023 g, 92%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 12.02$ (s, 1H), 11.59 (s, 1H), 7.82 (m, 1H), 7.31 (s, 2H), 6.55 (s, 1H), 3.00 (q, 2H, $J =$

6.4 Hz), 2.37 (t, 2H, $J=7.2$ Hz), 2.07 (t, 2H, $J=7.2$ Hz), 1.73 (m, 2H), 1.35 (m, 2H), 1.23 (brs, 26H), 0.85 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=171.27$, 146.67, 126.38, 108.67, 38.38, 34.36, 31.25, 29.10, 28.99, 28.96, 28.70, 28.66, 26.39, 23.83, 23.58, 22.05, 13.91 ppm; HRMS (ESI): m/z: calcd for $C_{23}H_{44}N_4O$: 392.3515 $[M]^+$; found: 392.3514.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-octadecyl-butyramide hydrochloride (12): By using the same general procedure as that used for the synthesis of **8**, **15** (0.028 g, 0.054 mmol) gave the title compound **12** (0.023 g, 94%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=12.02$ (s, 1H), 11.60 (s, 1H), 7.82 (s, 1H), 7.31 (s, 2H), 6.55 (s, 1H), 3.00 (q, 2H, $J=$ 6.6 Hz), 2.37 (t, 2H, $J=6.6$ Hz), 2.06 (t, 2H, $J=7.2$ Hz), 1.73 (m, 2H), 1.35 (m, 2H), 1.23 (brs, 30H), 0.85 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=171.29$, 146.68, 126.38, 108.70, 38.40, 34.38, 31.29, 29.14, 29.02, 28.74, 28.70, 26.42, 23.85, 23.60, 22.09, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{25}H_{48}N_4O$: 420.3828 $[M]^+$; found: 420.3839.

Deletion of the amide bond

1-Bromo-undecan-2-one: Decanoyl chloride (2.25 mL, 11.0 mmol) was dissolved into anhydrous dichloromethane (10 mL) and added dropwise to a 0°C solution of CH_2N_2 (33.0 mmol generated from Diazald/KOH) in diethyl ether (100 mL). This solution was stirred at 0°C for 1.5 h, after which time, the reaction was quenched by the dropwise addition of 48% HBr (4.0 mL). The reaction mixture was diluted with dichloromethane (25 mL) and immediately washed with sat. $NaHCO_3$ (3×25 mL) and brine (2×25 mL) before being dried ($MgSO_4$), filtered, and concentrated. The crude oil was purified by flash column chromatography (0–8% Et_2O/hexanes) to give the title compound (2.31 g, 84%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=4.32$ (s, 2H), 2.56 (t, 2H, $J=$ 7.2 Hz), 1.47 (m, 2H), 1.23 (brs, 12H), 0.85 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=201.70$, 39.08, 36.86, 31.29, 28.86, 28.80, 28.67, 28.43, 23.21, 22.12, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{11}H_{21}BrO$: 248.0776 $[M]^+$; found: 248.0787.

1-Bromo-tridecan-2-one: By using the same general procedure as that used for 1-bromo-undecan-2-one, dodecanoyl chloride (4.72 mL, 19.9 mmol) gave a crude oil that was purified by flash column chromatography (0–10% Et_2O/hexanes) to give the title compound (4.59 g, 83%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=4.32$ (s, 2H), 2.56 (t, 2H, $J=7.2$ Hz), 1.47 (m, 2H), 1.24 (brs, 16H), 0.85 ppm (t, 3H, $J=$ 6.6 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=201.71$, 38.96, 36.88, 31.32, 29.02, 28.90, 28.79, 28.73, 28.43, 23.21, 22.12, 13.97 ppm; HRMS (ESI): m/z: calcd for $C_{13}H_{25}BrO$: 276.1089 $[M]^+$; found: 276.1098.

1-Bromo-pentadecan-2-one: By using the same general procedure as that used for 1-bromo-undecan-2-one, tetradecanoyl chloride (2.97 mL, 11.0 mmol) gave a crude oil that was purified by flash column chromatography (0–8% Et_2O/hexanes) to give the title compound (3.08 g, 92%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 60°C): $\delta=4.27$ (s, 2H), 2.57 (t, 2H, $J=7.2$ Hz), 1.50 (m, 2H), 1.25 (brs, 20H), 0.86 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 60°C): $\delta=201.43$, 38.93, 36.10, 31.03, 28.77, 28.74, 28.71, 28.60, 28.49, 28.42, 28.20, 23.04, 21.80, 13.60 ppm; HRMS (ESI): m/z: calcd for $C_{15}H_{29}BrO$: 304.1402 $[M]^+$; found: 304.1415.

1-Bromo-heptadecan-2-one: By using the same general procedure as that used for 1-bromo-undecan-2-one, hexadecanoyl chloride (3.33 mL, 11.0 mmol) gave a crude oil that was purified by flash column chromatography (0–8% Et_2O/hexanes) to give the title compound (3.18 g, 87%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 75°C): $\delta=4.25$ (s, 2H), 2.58 (t, 2H, $J=7.2$ Hz), 1.51 (m, 2H), 1.26 (brs, 24H), 0.87 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 75°C): $\delta=201.34$, 38.87, 35.83, 30.92, 28.64, 28.48, 28.36, 28.29, 28.11, 22.97, 21.68, 13.46 ppm; HRMS (ESI): m/z: calcd for $C_{17}H_{33}BrO$: 332.1715 $[M]^+$; found: 332.1725.

1-Bromo-nonadecan-2-one: By using the same general procedure as that used for 1-bromo-undecan-2-one, octadecanoyl chloride (3.30 g, 11.0 mmol) gave a crude solid that was triturated with a 1:1 EtOAc/CH_2Cl_2 mixture and filtered to give the title compound (3.61 g, 91%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 80°C): $\delta=4.22$ (s, 2H), 2.58 (t, 2H, $J=7.2$ Hz), 1.52 (m, 2H), 1.26 (brs, 28H), 0.87 ppm (t, 3H, $J=6.6$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 61°C): $\delta=201.41$, 38.91, 36.06, 31.02, 28.76, 28.58, 28.47, 28.39, 28.19, 23.03, 21.78, 13.58 ppm; HRMS (ESI): m/z: calcd for $C_{19}H_{37}BrO$: 360.2028 $[M]^+$; found: 360.2036.

(*Z*,*Z*)-1-Bromo-nonadeca-10,13-dien-2-one: By using the same general procedure as that used for 1-bromo-undecan-2-one, octadecanoyl chloride (1.00 g, 3.34 mmol) gave a crude oil that was purified by flash column chromatography (0–8% Et_2O/hexanes) to give the title compound (0.940 g, 78%) as a colorless oil. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=5.32$ (m, 4H), 4.31 (s, 2H), 2.74 (t, 2H, $J=5.4$ Hz), 2.57 (t, 2H, $J=4.2$ Hz), 2.02 (q, 4H, $J=6.6$ Hz), 1.48 (quint., 2H, $J=6.6$ Hz), 1.27 (brs, 14H), 0.86 ppm (t, 3H, $J=6.6$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=201.62$, 129.70, 129.67, 127.74, 127.72, 39.05, 36.72, 30.89, 28.99, 28.72, 28.63, 28.50, 28.40, 26.60, 25.20, 23.18, 21.96, 13.89 ppm; HRMS (ESI): m/z: calcd for $C_{19}H_{33}BrO$: 356.1715 $[M]^+$; found: 356.1716.

(*Z*)-1-Bromo-octadec-10-en-2-one: By using the same general procedure as that used for 1-bromo-undecan-2-one, octadecanoyl chloride (3.30 g, 11.0 mmol) gave a crude oil that was purified by flash column chromatography (0–20% Et_2O/hexanes) to give the title compound (3.89 g, 98%) as a colorless oil. The product solidified upon standing. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=5.31$ (t, 2H, $J=4.2$ Hz), 4.29 (s, 2H), 2.56 (t, 2H, $J=6.9$ Hz), 1.97 (q, 4H, $J=6.0$ Hz), 1.47 (t, 2H, $J=6.6$ Hz), 1.24 (brs, 20H), 0.85 ppm (t, 3H, $J=6.6$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=201.53$, 129.56, 129.53, 38.95, 38.66, 36.69, 31.32, 29.14, 29.12, 28.89, 28.74, 28.70, 28.65, 28.52, 28.44, 26.59, 23.20, 22.12, 13.91 ppm; HRMS (ESI): m/z: calcd for $C_{19}H_{35}BrO$: 358.1871 $[M]^+$; found: 358.1866.

(*E*)-1-Bromo-9-methyl-dec-7-en-2-one: By using the same general procedure as that used for 1-bromo-undecan-2-one, octadecanoyl chloride (1.50 g, 7.95 mmol) gave a crude oil that was purified by flash column chromatography (0–10% Et_2O/hexanes) to give the title compound (1.92 g, 98%) as an amber oil. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=5.34$ (m, 2H), 4.31 (s, 2H), 2.57 (t, 2H, $J=7.2$ Hz), 2.20 (m, 1H), 1.93 (q, 2H, $J=6.0$ Hz), 1.48 (m, 2H), 1.28 (m, 2H), 0.92 ppm (d, 6H, $J=6.6$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=201.56$, 137.41, 126.44, 38.89, 36.75, 31.63, 30.38, 28.37, 22.69, 22.49 ppm; HRMS (ESI): m/z: calcd for $C_{11}H_{19}BrO$: 246.0619 $[M]^+$; found: 246.0614.

2-Amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester: 1-Bromo-undecan-2-one (0.748 g, 3.00 mmol), Boc-guanidine (1.40 g, 9.00 mmol), and NaI (0.900 g, 6.00 mmol) were dissolved in DMF (15 mL) and allowed to stir at room temperature. After 72 h, the DMF was removed under reduced pressure and the residue was taken up in ethyl acetate (50 mL) and washed with water (3×25 mL) and brine (25 mL) before being dried (Na_2SO_4), filtered, and evaporated to dryness. The resulting oil was purified by flash column chromatography (0–50% EtOAc/hexanes) to give the title compound (0.408 g, 44%) as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=6.47$ (s, 1H), 6.41 (s, 2H), 2.21 (t, 2H, $J=7.2$ Hz), 1.51 (s, 9H), 1.47 (m, 2H), 1.22 (brs, 12H), 0.84 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=149.93$, 148.98, 138.91, 105.44, 83.88, 31.32, 28.99, 28.92, 28.74, 27.83, 27.72, 27.48, 22.13, 13.93 ppm; HRMS (ESI): m/z: calcd for $C_{17}H_{23}N_3O_2$: 309.2416 $[M]^+$; found: 309.2426.

2-Amino-4-undecyl-imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester, 1-bromo-tridecan-2-one (4.00 g, 14.4 mmol) gave a crude oil that was purified by flash column chromatography (0–30% EtOAc/hexanes) to give the title compound (2.23 g, 46%) as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=6.49$ (s, 1H), 6.37 (s, 2H), 2.22 (brs, 2H), 1.52 (s, 9H), 1.48 (m, 2H), 1.23 (brs, 16H), 0.86 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=149.87$, 148.97, 138.94, 105.52, 83.95, 31.31, 29.01, 28.87, 28.72, 27.80, 27.70, 27.51, 22.11, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{19}H_{35}N_3O_2$: 337.2729 $[M]^+$; found: 337.2730.

2-Amino-4-tridecyl-imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester, 1-bromo-pentadecan-2-one (0.916 g, 3.00 mmol) gave a crude oil that was purified by flash column chromatography (0–60% EtOAc/hexanes) to give the title compound (0.473 g, 43%) as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 75°C): $\delta=$ 6.48 (s, 1H), 6.22 (s, 2H), 2.24 (t, 2H, $J=7.2$ Hz), 1.54 (s, 9H), 1.50 (m,

2H), 1.25 (brs, 20H), 0.86 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 75 °C): $\delta=149.45$, 148.69, 138.82, 105.28, 83.71, 30.92, 28.64, 28.59, 28.48, 28.36, 28.30, 27.51, 27.43, 27.27, 21.67, 13.44 ppm; HRMS (ESI): m/z: calcd for $C_{21}H_{39}N_3O_2$: 365.3042 $[M]^+$; found: 365.3048.

2-Amino-4-pentadecyl-imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester, 1-bromo-heptadecan-2-one (1.00 g, 3.00 mmol) gave a crude oil that was purified by flash column chromatography (0–60% EtOAc/hexanes) to give the title compound (0.628 g, 53%) as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 75 °C): $\delta=6.49$ (s, 1H), 6.20 (s, 2H), 2.24 (t, 2H, $J=7.2$ Hz), 1.54 (s, 9H), 1.50 (m, 2H), 1.25 (brs, 24H), 0.86 ppm (t, 3H, $J=6.4$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 75 °C): $\delta=149.42$, 148.67, 138.82, 105.31, 83.73, 30.90, 28.61, 28.56, 28.45, 28.34, 28.27, 27.49, 27.42, 27.28, 21.65, 13.43 ppm; HRMS (ESI): m/z: calcd for $C_{23}H_{43}N_3O_2$: 393.3355 $[M]^+$; found: 393.3362.

2-Amino-4-heptadecyl-imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester, 1-bromo-nonadecan-2-one (1.14 g, 3.15 mmol), with additional DMF (25 mL) for solubility, gave a crude oil that was purified by trituration (MeOH/H$_2$O 1:20) to give the title compound (1.10 g, 83%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): $\delta=6.49$ (s, 1H), 6.19 (s, 2H), 2.24 (t, 2H, $J=7.2$ Hz), 1.54 (s, 9H), 1.50 (m, 2H), 1.25 (brs, 28H), 0.87 ppm (t, 3H, $J=6.8$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 80 °C): $\delta=149.45$, 148.71, 138.85, 105.33, 83.76, 30.93, 28.64, 28.49, 28.38, 28.30, 27.62, 27.52, 27.45, 27.30, 21.68, 13.44 ppm; HRMS (ESI): m/z: calcd for $C_{25}H_{47}N_3O_2$: 421.3668 $[M]^+$; found: 421.3678.

(*Z*,*Z*)-2-Amino-4-heptadeca-8,11-dienyl-imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester, 1-bromo-nonadeca-10,13-dien-2-one (0.660 g, 1.85 mmol) gave a crude oil that was purified by flash column chromatography (0–30% EtOAc/hexanes) to give the title compound (0.382 g, 50%) as a dark-brown oil; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=6.47$ (s, 1H), 6.37 (s, 2H), 5.31 (m, 4H), 2.72, (t, 2H, $J=6.0$ Hz), 2.21 (t, 2H, $J=7.2$ Hz), 2.00 (q, 4H, $J=6.8$ Hz), 1.52 (s, 9H), 1.48 (m, 2H), 1.25 (brs, 14H), 0.84 ppm (t, 3H, $J=6.4$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=149.88$, 148.96, 138.92, 129.69, 127.73, 105.45, 83.92, 30.92, 29.04, 28.74, 28.71, 28.61, 27.80, 27.71, 27.49, 26.62, 25.21, 21.98, 13.90 ppm; HRMS (ESI): m/z: calcd for $C_{25}H_{43}N_3O_2$: 417.3355 $[M]^+$; found: 417.3360.

(*Z*)-2-Amino-4-heptadec-8-enyl-imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester, 1-bromo-octadec-10-en-2-one (1.00 g, 2.78 mmol) gave a crude oil that was purified by flash column chromatography (20–100% EtOAc/hexanes) to give the title compound (0.578 g, 50%) as pale-yellow oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=6.48$ (s, 1H), 6.36 (s, 2H), 5.31 (t, 2H, $J=4.4$ Hz), 2.21 (t, 2H, $J=7.2$ Hz), 1.97 (m, 4H), 1.52 (s, 9H), 1.48 (m, 2H), 1.25 (brs, 10H), 1.23 (brs, 10H), 0.84 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=149.88$, 148.96, 138.92, 129.65, 105.49, 83.96, 31.30, 29.11, 28.85, 28.72, 28.60, 28.55, 27.80, 27.72, 27.51, 26.57, 22.12, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{25}H_{45}N_3O_2$: 419.3512 $[M]^+$; found: 419.3515.

(*E*)-2-Amino-4-(7-methyl-oct-5-enyl)imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester, 1-bromo-9-methyl-dec-7-en-2-one (1.00 g, 4.05 mmol) gave a crude oil that was purified by flash column chromatography (0–80% EtOAc/hexanes) to give the title compound (0.594 g, 48%) as a yellow oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=6.49$ (s, 1H), 6.38 (s, 2H), 5.34 (m, 2H), 2.22 (m, 3H), 1.94 (q, 2H, $J=6.4$ Hz), 1.52 (s, 9H), 1.48 (m, 2H), 1.31 (m, 2H), 0.92 ppm (d, 6H, $J=6.8$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=149.89$, 148.95, 138.88, 137.27, 126.76, 105.55, 83.98, 31.80, 30.40, 28.76, 27.51, 27.36, 22.56 ppm; HRMS (ESI): m/z: calcd for $C_{17}H_{29}N_3O_2$: 307.2260 $[M]^+$; found: 307.2262.

4-Nonyl-1*H*-imidazol-2-ylamine hydrochloride (18): A solution of 2-amino-4-nonyl-imidazole-1-carboxylic acid *tert*-butyl ester (0.200 g, 0.650 mmol) in anhydrous dichloromethane (6 mL) was cooled to 0 °C. TFA (6 mL) was charged into the flask and the reaction stirred for 6 h. After this time, the reaction was evaporated to dryness and toluene (2 mL) was added. Again the mixture was concentrated and the process repeated. The resulting TFA salt was dissolved in dichloromethane (1 mL) and 2 M HCl in diethyl ether (1.0 mL) was added followed by cold diethyl ether (8 mL). The precipitate was collected by filtration and washed with diethyl ether (3 mL) to yield the target compound **18** (0.159 g, 99%) as a brown amorphous solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.19$ (s, 1H), 11.76 (s, 1H), 7.35 (s, 2H), 6.54 (s, 1H), 2.37 (t, 2H, $J=7.2$ Hz), 1.49 (m, 2H), 1.22 (brs, 12H), 0.83 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=146.80$, 126.85, 108.42, 31.29, 28.91, 28.69, 28.35, 27.61, 23.99, 22.12, 13.97 ppm; HRMS (ESI): m/z: calcd for $C_{21}H_{23}N_3$: 209.1892 $[M]^+$; found: 209.1901.

4-Undecyl-1*H*-imidazol-2-ylamine hydrochloride (19): By using the same general procedure as that used for the synthesis of **18**, 2-amino-4-undecyl-imidazole-1-carboxylic acid *tert*-butyl ester (2.23 g, 6.60 mmol) gave the title compound **19** (1.80 g, 99%) as a brown amorphous solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.19$ (s, 1H), 11.72 (s, 1H), 7.34 (s, 2H), 6.54 (s, 1H), 2.37 (d, 2H, $J=5.6$ Hz), 1.50 (m, 2H), 1.24 (brs, 16H), 0.85 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=146.76$, 126.81, 108.39, 31.32, 29.05, 29.02, 28.96, 28.76, 28.69, 28.36, 27.61, 27.61, 23.99, 22.12, 13.98 ppm; HRMS (ESI): m/z: calcd for $C_{14}H_{28}N_3$: 237.2205 $[M]^+$; found: 237.2209.

4-Tridecyl-1*H*-imidazol-2-ylamine hydrochloride (20): By using the same general procedure as that used for the synthesis of **18**, 2-amino-4-tridecyl-imidazole-1-carboxylic acid *tert*-butyl ester (0.200 g, 0.550 mmol) gave the title compound **20** (0.158 g, 96%) as a brown amorphous solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.19$ (s, 1H), 11.68 (s, 1H), 7.34 (s, 2H), 6.53 (s, 1H), 2.38 (t, 2H, $J=7.2$ Hz), 1.50 (m, 2H), 1.23 (brs, 20H), 0.84 ppm (t, 3H, $J=7.2$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=146.77$, 126.80, 108.36, 31.31, 29.08, 29.05, 28.96, 28.73, 28.69, 28.36, 27.61, 23.98, 22.11, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{16}H_{31}N_3$: 265.2518 $[M]^+$; found: 265.2529.

4-Pentadecyl-1*H*-imidazol-2-ylamine hydrochloride (21): By using the same general procedure as that used for the synthesis of **18**, 2-amino-4-pentadecyl-imidazole-1-carboxylic acid *tert*-butyl ester (0.200 g, 0.510 mmol) gave the title compound **21** (0.167 g, 99%) as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.18$ (s, 1H), 11.65 (s, 1H), 7.32 (s, 2H), 6.53 (s, 1H), 2.38 (t, 2H, $J=7.2$ Hz), 1.50 (m, 2H), 1.23 (brs, 24H), 0.84 ppm (t, 3H, $J=7.6$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=146.73$, 126.77, 108.33, 31.31, 29.08, 29.03, 28.98, 28.74, 28.71, 28.39, 27.63, 23.99, 22.12, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{18}H_{35}N_3$: 293.2831 $[M]^+$; found: 293.2844.

4-Heptadecyl-1*H*-imidazol-2-ylamine hydrochloride (22): By using the same general procedure as that used for the synthesis of **18**, 2-amino-4-heptadecyl-imidazole-1-carboxylic acid *tert*-butyl ester (0.400 g, 0.950 mmol) gave the title compound **22** (0.320 g, 94%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.10$ (brs, 1H), 11.70 (brs, 1H), 7.33 (s, 2H), 6.54 (s, 1H), 2.37 (t, 2H, $J=7.6$ Hz), 1.50 (m, 2H), 1.23 (brs, 28H), 0.84 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=146.75$, 126.80, 108.36, 31.31, 29.06, 28.76, 28.39, 27.62, 24.00, 22.12, 13.96 ppm; HRMS (ESI): m/z: calcd for $C_{20}H_{39}N_3$: 321.3144 $[M]^+$; found: 321.3146.

(*Z*,*Z*)-4-Heptadeca-8,11-dienyl-1*H*-imidazol-2-ylamine hydrochloride (23): By using the same general procedure as that used for the synthesis of **18**, (*Z*,*Z*)-2-amino-4-heptadeca-8,11-dienyl-imidazole-1-carboxylic acid *tert*-butyl ester (0.323 g, 0.770 mmol) gave the title compound **23** (0.270 g, 99%) as a brown oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.10$ (s, 1H), 11.64 (s, 1H), 7.32 (s, 2H), 6.54 (s, 1H), 5.33 (m, 4H), 2.73 (t, 2H, $J=6.0$ Hz), 2.38 (t, 2H, $J=7.2$ Hz), 2.02 (q, 4H, $J=5.6$ Hz), 1.50 (m, 2H), 1.27 (brs, 14H), 0.85 ppm (t, 3H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=146.69$, 129.70, 127.74, 126.84, 108.42, 30.91, 29.04, 28.74, 28.56, 28.36, 27.61, 26.63, 25.22, 23.98, 21.98, 13.94 ppm; HRMS (ESI): m/z: calcd for $C_{20}H_{35}N_3$: 317.2831 $[M]^+$; found: 317.2836.

(*Z*)-4-Heptadec-8-enyl-1*H*-imidazol-2-ylamine hydrochloride (24): By using the same general procedure as that used for the synthesis of **18**, (*Z*)-2-amino-4-heptadec-8-enyl-imidazole-1-carboxylic acid *tert*-butyl

ester (0.528 g, 1.26 mmol) gave the title compound **24** (0.447 g, 100%) as a brown oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=12.10 (s, 1H), 11.75 (s, 1H), 7.35 (s, 2H), 6.52 (s, 1H), 5.31 (m, 2H), 2.37 (t, 2H, J=7.2 Hz), 1.97 (m, 4H), 1.50 (m, 2H), 1.25 (brs, 10H), 1.22 (brs, 10H), 0.84 ppm (t, 3H, J=6.0 Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=146.82, 129.64, 129.61, 126.79, 108.35, 31.31, 29.14, 29.12, 28.86, 28.72, 28.61, 28.58, 28.39, 27.62, 26.62, 26.59, 24.00, 22.12, 13.95 ppm; HRMS (ESI): m/z: calcd for $C_{20}H_{37}N_3$: 319.2988 $[M]^+$; found: 319.3009.

(*E*)-4-(7-Methyl-oct-5-enyl)-1*H*-imidazol-2-ylamine hydrochloride (24): By using the same general procedure as that used for the synthesis of **18**, (*E*)-2-amino-4-(7-methyl-oct-5-enyl)-imidazole-1-carboxylic acid *tert*-butyl ester (0.479 g, 1.56 mmol) gave the title compound **24** (0.350 g, 93%) as a brown oil. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=12.18 (s, 1H), 11.71 (s, 1H), 7.34 (s, 2H), 6.54 (s, 1H), 5.35 (m, 2H), 2.39 (t, 2H, J=7.6 Hz), 2.20 (m, 1H), 1.94 (q, 2H, J=6.4 Hz), 1.50 (quint., 2H, J=7.6 Hz), 0.92 ppm (d, 6H, J=6.8 Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=146.77, 137.46, 126.74, 126.53, 108.44, 31.53, 30.38, 28.33, 27.13, 23.81, 22.53 ppm; HRMS (ESI): m/z: calcd for $C_{12}H_{21}N_3$: 207.1736 $[M]^+$; found: 207.1738.

Linker modification

Succinic acid monobenzyl ester: Succinic anhydride (5.00 g, 50.0 mmol) was dissolved in anhydrous dichloromethane (40 mL). Benzyl alcohol (5.69 mL, 55.0 mmol), triethylamine (7.50 mL, 55.0 mmol), and a catalytic amount of DMAP were added to this solution. The resulting clear solution was stirred at ambient temperature for 15 h, after which time, all volatiles were removed by rotary evaporation. The crude residue was taken up in diethyl ether (200 mL) and was washed with 2 N NaOH (2×75 mL). The aqueous extracts were carefully acidified to pH 2 with concentrated HCl and then extracted with diethyl ether (2×100 mL) and subsequently dried (Mg_2SO_4), filtered, and concentrated to afford the title compound (9.06 g, 87%) as a white solid with no further purification needed. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=12.26 (s, 1H), 7.32 (m, 5H), 5.08 (s, 2H), 2.56 (m, 2H), 2.49 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=173.53, 172.13, 136.26, 128.48, 128.02, 127.88, 65.56, 28.80, 28.73 ppm; HRMS (ESI): m/z: calcd for $C_{11}H_{12}O_4$: 208.0736 $[M]^+$; found: 208.0736.

5-Bromo-4-oxo-pentanoic acid benzyl ester: Succinic acid monobenzyl ester (2.00 g, 9.60 mmol) was dissolved in anhydrous dichloromethane (30 mL) at 0 °C and a catalytic amount of DMF was added. Oxalyl chloride (2.50 mL, 28.8 mmol) was added dropwise to this solution, and the resulting mixture was then warmed to room temperature. After 1 h, the solvent and excess oxalyl chloride were removed under reduced pressure. The resulting solid was dissolved into anhydrous dichloromethane (10 mL) and added dropwise to a 0 °C solution of CH_2N_2 (28.8 mmol generated from Diazald/KOH) in diethyl ether (75 mL). This solution was stirred at 0 °C for 1.5 h, after which time, the reaction was quenched by the dropwise addition of 48% HBr (3.5 mL). The reaction mixture was diluted with dichloromethane (25 mL) and immediately washed with sat. $NaHCO_3$ (3×25 mL) and brine (2×25 mL) before being dried ($MgSO_4$), filtered, and concentrated. The resulting yellow oil (2.59 g, 95%) was carried onto the next step without further purification. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.36 (m, 5H), 5.08 (s, 2H), 4.39 (s, 2H), 2.88 (t, 2H, J=6.0 Hz), 2.60 ppm (t, 2H, J=6.0 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=200.35, 171.83, 136.06, 128.36, 127.94, 127.77, 65.54, 36.33, 34.20, 27.77 ppm; HRMS (ESI): m/z: calcd for $C_{12}H_{13}BrO_3$: 284.0048 $[M]^+$; found: 284.0056.

2-Amino-4-(2-benzyloxycarbonyl-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester: 5-bromo-4-oxo-pentanoic acid benzyl ester (1.00 g, 3.51 mmol) and Boc-guanidine (1.66 g, 10.5 mmol) were dissolved in DMF (10 mL) and allowed to stir at room temperature. After 48 h, the DMF was removed under reduced pressure and the residue was taken up in ethyl acetate (50 mL) and washed with water (3×25 mL) and brine (25 mL) before being dried (Na_2SO_4), filtered, and evaporated to dryness. The resulting oil was purified by flash column chromatography (20–100% EtOAc/hexanes) to give the title compound (0.725 g, 60%) as a yellow solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.32 (m, 5H), 6.54 (s, 1H), 6.44 (s, 2H), 5.09 (s, 2H), 2.58 (m, 4H), 1.52 ppm (s, 9H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=172.23, 150.00, 148.88, 137.21, 136.27, 128.36, 127.89, 127.74, 105.92, 84.12, 65.33, 32.31, 27.48, 23.31 ppm; HRMS (ESI): m/z: calcd for $C_{18}H_{23}N_3O_4$: 345.1689 $[M]^+$; found: 345.1689.

2-Amino-4-(2-carboxy-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester (26): 2-Amino-4-(2-benzyloxycarbonyl-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester (2.00 g, 5.79 mmol) was added to a solution of anhydrous THF (50 mL) and 10% Pd/C (0.200 g). Air was removed from the system and the reaction was back flushed with hydrogen. This process was repeated three times before setting the reaction under a hydrogen balloon at atmospheric pressure and temperature for 1 h. After this time, the reaction was filtered through a Celite pad and the filter cake was washed with THF (10 mL). The filtrate was concentrated under reduced pressure to afford the title compound **26** (1.35 g, 92%) as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=6.54 (s, 1H), 6.44 (s, 2H), 2.46 (m, 4H), 1.53 ppm (s, 9H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=173.98, 149.96, 148.89, 137.55, 105.77, 84.19, 32.51, 27.52, 23.30 ppm; HRMS (ESI): m/z: calcd for $C_{11}H_{17}N_3O_4$: 255.1219 $[M]^+$; found: 255.1219.

7-Bromo-6-oxo-heptanoic acid benzyl ester: By using the same general procedure as that used for 5-bromo-4-oxo-pentanoic acid benzyl ester, hexanedioic acid monobenzyl ester[37] (2.60 g, 11.0 mmol) gave a crude amber oil (2.65 g, 77%) that was used in the next steps without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.27 (m, 5H), 5.03 (s, 2H), 3.77 (s, 2H), 2.58 (m, 2H), 2.30 (m, 2H), 1.58 ppm (q, 4H, J=3.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=201.80, 173.23, 136.14, 128.75, 128.41, 77.66, 77.23, 76.81, 66.41, 39.48, 34.30, 34.08, 24.35, 23.36 ppm; HRMS (ESI): m/z: calcd for $C_{14}H_{17}BrO_3$: 312.0361 $[M]^+$; found: 312.0367.

2-Amino-4-(4-benzyloxycarbonyl-butyl)imidazole-1-carboxylic acid *tert*-butyl ester: By using the same general procedure as that used for 2-amino-4-(2-benzyloxycarbonyl-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester, 7-bromo-6-oxo-heptanoic acid benzyl ester (1.50 g, 4.79 mmol) gave a crude oil that was purified by flash column chromatography (40–100% EtOAc/hexanes) to give the title compound (0.896 g, 50%) as an amber oil. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=7.35 (m, 5H), 6.51 (s, 1H), 6.38 (s, 2H), 5.07 (s, 2H), 2.36 (t, 2H, J=6.9 Hz), 2.24 (t, 2H, J=6.9 Hz), 1.52 ppm (m, 13H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=172.73, 149.84, 148.92, 138.54, 136.27, 128.38, 127.91, 127.85, 105.64, 84.00, 65.25, 33.26, 27.48, 27.26, 27.18, 24.08 ppm; HRMS (ESI): m/z: calcd for $C_{20}H_{27}N_3O_4$: 373.2002 $[M]^+$; found: 373.2006.

2-Amino-4-(4-carboxy-butyl)imidazole-1-carboxylic acid *tert*-butyl ester (27): By using the same general procedure as that used for **26**, 2-amino-4-(4-benzyloxycarbonyl-butyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.840 g, 2.25 mmol) gave the title compound **27** (0.538 g, 84%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=6.52 (s, 1H), 6.40 (s, 2H), 2.20 (m, 4H), 1.50 ppm (m, 13H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=174.56, 149.88, 148.92, 138.56, 105.68, 84.03, 33.59, 27.50, 27.32, 24.19 ppm; HRMS (ESI): m/z: calcd for $C_{13}H_{21}N_3O_4$: 283.1532 $[M]^+$; found: 283.1532.

2-Amino-4-(2-hexylcarbamoyl-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, the title compound was formed as a yellow foam (0.021 g, 8%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.57 (s, 1H), 6.06 (s, 1H), 5.67 (brs, 2H), 3.20 (q, 2H, J=6.9 Hz), 2.69 (t, 2H, J=7.2 Hz), 2.47 (t, 2H, J=7.2 Hz), 1.57 (s, 9H), 1.43 (m, 2H), 1.25 (brs, 6H), 0.87 ppm (t, 3H, J=6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=172.58, 150.20, 149.58, 137.38, 107.53, 85.10, 39.63, 35.87, 31.69, 29.69, 28.15, 26.73, 24.37, 22.75, 14.23 ppm; HRMS (ESI): m/z: calcd for $C_{17}H_{31}N_4O_3$: 338.2318 $[M]^+$; found: 338.2323.

2-Amino-4-(4-hexylcarbamoyl-butyl)imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, the title compound was formed as a colorless oil (0.044 g, 17%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=7.73 (t, 1H, J=5.4 Hz), 6.50 (s, 1H), 6.38 (s, 2H), 3.00 (q, 2H, J=6.6 Hz), 2.23 (m, 2H), 2.03 (m, 2H), 1.53 (s, 9H), 1.47 (m, 4H), 1.35 (m, 2H), 1.23 (m, 4H), 0.84 ppm (t, 3H, J=6.6 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=171.78, 149.81, 148.92, 138.70, 105.60, 84.00, 38.31, 35.25, 30.95, 29.09, 27.49, 27.39, 26.03, 25.03, 22.01, 13.85 ppm; HRMS (ESI): m/z: calcd for $C_{19}H_{34}N_4O_3$: 366.2631 $[M]^+$; found: 366.2632.

2-Amino-4-{2-[2-(4-bromo-phenyl)ethylcarbamoyl]ethyl}imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, the title compound was formed as an off-white solid (0.019 g, 6%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39 (d, 2H, J = 8.1 Hz), 7.03 (d, 2H, J = 8.4 Hz), 6.58 (s, 1H), 6.41 (m, 1H), 3.45 (q, 2H, J = 6.3 Hz), 2.72 (q, 2H, J = 6.9 Hz), 2.47 (t, 4H, J = 6.9 Hz), 1.59 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.19, 150.03, 148.93, 138.20, 133.98, 131.78, 130.66, 120.44, 107.50, 86.48, 40.53, 35.27, 35.04, 28.12, 23.12 ppm; HRMS (ESI): m/z: calcd for $C_{19}H_{25}BrN_4O_3$: 436.1110 $[M]^+$; found: 436.1112.

2-Amino-4-[2-(4-phenyl-butylcarbamoyl)ethyl]imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, the title compound was formed as a yellow foam (0.081 g, 27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26 (t, 2H, J = 7.6 Hz), 7.15 (m, 2H), 6.55 (s, 1H), 6.28 (brs, 1H), 5.75 (brs, 2H), 3.22 (q, 2H, J = 6.4 Hz), 2.66 (t, 2H, J = 7.2 Hz), 2.59 (t, 2H, J = 7.6 Hz), 2.45 (t, 2H, J = 7.2 Hz), 1.55 (m, 11H), 1.47 ppm (m, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.19, 149.79, 148.86, 142.08, 138.12, 128.21, 128.17, 125.58, 105.58, 84.01, 38.09, 34.73, 33.98, 28.77, 28.27, 27.47, 24.01 ppm; HRMS (ESI): m/z: calcd for $C_{21}H_{31}N_4O_3$: 386.2318 $[M]^+$; found: 386.2319.

3-(2-Amino-1*H*-imidazol-4-yl)-*N*-hexyl-propionamide hydrochloride (28): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-(2-hexylcarbamoyl-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.020 g, 0.062 mmol) gave the title compound **28** (0.017 g, 100%) as a tan-colored foam. ^{1}H NMR (300 MHz, CD_3OD): δ = 6.38 (s, 1H), 3.05 (t, 2H, J = 6.6 Hz), 2.66 (t, 2H, J = 6.6 Hz), 2.37 (t, 2H, J = 6.6 Hz), 1.36 (m, 2H), 1.19 (brs, 6H), 0.79 ppm (m, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ = 174.12, 148.60, 128.03, 110.15, 40.57, 35.27, 32.75, 30.48, 27.77, 23.73, 21.72, 14.45 ppm; HRMS (ESI): m/z: calcd for $C_{12}H_{22}N_4O$: 238.1794 $[M]^+$; found: 238.1795.

5-(2-Amino-1*H*-imidazol-4-yl)pentanoic acid hexylamide hydrochloride (29): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-(4-hexylcarbamoyl-butyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.036 g, 0.098 mmol) gave the title compound **29** (0.029 g, 100%) as a colorless oil. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.99 (s, 1H), 11.55 (s, 1H), 7.78 (m, 1H), 7.29 (s, 2H), 6.55 (s, 1H), 3.00 (q, 2H, J = 6.9 Hz), 2.39 (brs, 2H), 2.06 (brs, 2H), 1.48 (brs, 4H), 1.36 (m, 2H), 1.28 (brs, 4H), 0.85 ppm (m, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.62, 146.61, 126.73, 108.51, 38.34, 34.92, 30.93, 29.07, 27.23, 26.03, 24.55, 23.71, 22.00, 13.85 ppm; HRMS (ESI): m/z: calcd for $C_{14}H_{26}N_4O$: 266.2107 $[M]^+$; found: 266.2109.

3-(2-Amino-1*H*-imidazol-4-yl)-*N*-[2-(4-bromo-phenyl)ethyl]propionamide hydrochloride (30): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-{2-[2-(4-bromo-phenyl)ethylcarbamoyl]ethyl}imidazole-1-carboxylic acid *tert*-butyl ester (0.019 g, 0.043 mmol) gave the title compound **30** (0.016 g, 100%) as a brown foam. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.29 (d, 2H, J = 7.8 Hz), 6.99 (d, 2H, J = 8.1 Hz), 6.33 (s, 1H), 3.27 (t, 2H, J = 7.2 Hz), 2.61 (m, 4H), 2.33 ppm (t, 2H, J = 6.6 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ = 174.20, 139.89, 132.63, 131.92, 127.98, 121.19, 110.14, 41.72, 35.95, 35.18, 21.62 ppm; HRMS (ESI): m/z: calcd for $C_{14}H_{17}BrN_4O$: 336.0586 $[M]^+$; found: 336.0591.

3-(2-Amino-1*H*-imidazol-4-yl)-*N*-(4-phenyl-butyl)propionamide hydrochloride (31): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-[2-(4-phenyl-butylcarbamoyl)ethyl]imidazole-1-carboxylic acid *tert*-butyl ester (0.080 g, 0.206 mmol) gave the title compound **31** (0.066 g, 100%) as a tan-colored foam. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.91 (s, 1H), 11.59 (s, 1H), 7.97 (m, 1H), 7.34 (s, 2H), 7.25 (m, 2H), 7.17 (d, 3H, J = 7.2 Hz), 6.50 (s, 1H), 3.05 (q, 2H, J = 6.0 Hz), 2.58 (m, 4H), 2.35 (t, 2H, J = 7.2 Hz), 1.53 (quint., 2H, J = 7.2 Hz), 1.38 ppm (quint., 2H, J = 7.2 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 170.62, 146.57, 142.06, 128.24, 128.19, 126.21, 125.61, 108.51, 38.24, 34.73, 33.46, 28.72, 28.30, 20.19 ppm; HRMS (ESI): m/z: calcd for $C_{16}H_{22}N_4O$: 286.1794 $[M]^+$; found: 286.1799.

Sliding the amide bond

2-Amino-4-(2-heptylcarbamoyl-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, 2-amino-4-(3-carboxy-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.115 g, 0.450 mmol) gave the title compound (0.044 g, 27%) as a tan-colored solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.78 (m, 1H), 6.50 (s, 1H), 6.38 (s, 2H), 3.00 (q, 2H, J = 5.6 Hz), 2.46 (t, 2H, J = 8.0 Hz), 2.28 (t, 2H, J = 7.2 Hz), 1.52 (s, 9H), 1.35 (m, 2H), 1.22 (brs, 8H), 0.85 ppm (m, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.18, 149.82, 148.91, 138.09, 105.63, 84.05, 38.37, 33.99, 31.23, 29.16, 28.42, 27.51, 26.32, 24.04, 22.06, 13.95 ppm; HRMS (ESI): m/z: calcd for $C_{18}H_{32}N_4O_3$: 352.2474 $[M]^+$; found: 352.2489.

2-Amino-4-(4-pentylcarbamoyl-butyl)imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, the title compound was formed as an amber oil (0.012 g, 5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.52 (s, 1H), 5.66 (s, 2H), 5.55 (m, 1H), 3.23 (q, 2H, J = 6.0 Hz), 2.38 (t, 2H, J = 6.6 Hz), 2.18 (t, 2H, J = 7.2 Hz), 1.66 (m, 4H), 1.59 (s, 9H), 1.50 (m, 2H), 1.29 (m, 4H), 0.89 ppm (t, 3H, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.07, 150.12, 149.63, 138.74, 106.99, 84.91, 39.70, 36.85, 29.91, 29.58, 29.30, 28.20, 28.14, 27.94, 25.55, 22.56, 14.18 ppm; HRMS (ESI): m/z: calcd for $C_{18}H_{32}N_4O_3$: 352.2474 $[M]^+$; found: 352.2482.

3-(2-Amino-1*H*-imidazol-4-yl)-*N*-heptyl-propionamide hydrochloride (32): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-(2-heptylcarbamoyl-ethyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.031 g, 0.088 mmol) gave the title compound **32** (0.025 g, 99%) as a tan-colored amorphous solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.88 (s, 1H), 11.56 (s, 1H), 7.93 (m, 1H), 7.33 (s, 2H), 6.51 (s, 1H), 3.02 (q, 2H, J = 6.4 Hz), 2.62 (t, 2H, J = 7.2 Hz), 2.35 (t, 2H, J = 7.2 Hz), 1.36 (m, 2H), 1.23 (brs, 8H), 0.86 ppm (t, 3H, J = 6.4 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 170.61, 146.56, 126.24, 108.55, 38.48, 33.48, 31.20, 29.06, 28.37, 26.33, 22.02, 20.20, 13.93 ppm; HRMS (ESI): m/z: calcd for $C_{13}H_{24}N_4O$: 252.1950 $[M]^+$; found: 252.1959.

5-(2-Amino-1*H*-imidazol-4-yl)pentanoic acid pentylamide hydrochloride (33): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-(4-pentylcarbamoyl-butyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.012 g, 0.034 mmol) gave the title compound **33** (0.009 g, 100%) as an amber oil. ^{1}H NMR (300 MHz, CD_3OD): δ = 6.50 (s, 1H), 3.15 (t, 2H, J = 6.9 Hz), 2.51 (t, 2H, J = 6.6 Hz), 2.22 (t, 2H, J = 6.9 Hz), 1.63 (m, 4H), 1.49 (m, 2H), 1.32 (m, 4H), 0.91 ppm (t, 3H, J = 6.9 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ = 176.25, 149.01, 129.29, 110.34, 40.89, 37.04, 30.72, 30.61, 29.28, 26.69, 25.65, 23.89, 14.82 ppm; HRMS (ESI): m/z: calcd for $C_{13}H_{24}N_4O$: 252.1950 $[M]^+$; found: 252.1951.

Increased substitution

2-Amino-4-[2-(1-hexyl-heptylcarbamoyl)ethyl]imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, **26** afforded an off-white solid (0.176 g, 51%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.46 (d, 1H, J = 8.7 Hz), 6.50 (s, 1H), 6.36 (s, 2H), 3.63 (m, 1H), 2.47 (t, 2H, J = 7.2 Hz), 2.28 (t, 2H, J = 7.2 Hz), 1.52 (s, 9H), 1.20 (m, 20H), 0.84 ppm (t, 6H, J = 7.2 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 170.79, 149.80, 148.93, 138.07, 105.66, 83.95, 47.74, 34.65, 33.98, 31.25, 28.63, 27.50, 25.44, 24.24, 22.06, 13.93 ppm; HRMS (ESI): m/z: calcd for $C_{24}H_{44}N_4O_3$: 436.3413 $[M]^+$; found: 436.3414.

2-Amino-4-[3-(1-hexyl-heptylcarbamoyl)propyl]imidazole-1-carboxylic acid *tert*-butyl ester: By using the general EDC/HOBt procedure, the title compound was formed as a tan-colored solid (0.035 g, 21%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.43 (d, 1H, J = 8.8 Hz), 6.48 (s, 1H), 6.38 (s, 2H), 3.66 (m, 1H), 2.21 (t, 2H, J = 7.2 Hz), 2.04 (t, 2H, J = 7.2 Hz), 1.71 (m, 2H), 1.52 (s, 9H), 1.23 (m, 20H), 0.84 ppm (m, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.31, 149.94, 148.94, 138.41, 105.72, 84.01, 47.68, 35.06, 34.67, 31.30, 31.11, 28.63, 27.51, 27.18, 25.52, 24.23, 22.06, 22.03, 13.95 ppm; HRMS (ESI): m/z: calcd for $C_{25}H_{46}N_4O_3$: 450.3570 $[M]^+$; found: 450.3562.

4-Dihexylcarbamoyl-butyric acid: Glutaric anhydride (1.00 g, 8.76 mmol) was dissolved in anhydrous dichloromethane (12 mL). Dihexylamine (2.27 mL, 9.64 mmol), triethylamine (1.30 mL, 9.64 mmol), and a catalytic amount of DMAP were added to this solution and the resulting clear solution was stirred at ambient temperature for 15 h, after which time, all volatiles were removed by rotary evaporation. The crude residue was partitioned between ethyl acetate (150 mL) and a 1 N HCl aqueous solution (100 mL). The organic layer was subsequently washed with 1 N HCl (3 × 50 mL), saturated $NaHCO_3$ (2 × 75 mL), and brine (1 × 50 mL). The organics were then dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness to afford the title compound (2.44 g, 93%) as a viscous oil that required no further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.28 (t, 2H, J = 7.5 Hz), 3.19 (t, 2H, J = 7.5 Hz), 2.41 (m, 4H), 1.95 (t, 2H, J = 6.9 Hz), 1.51 (m, 4H), 1.27 (brs, 12H), 0.88 ppm (m, 6H);

^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.62, 172.32, 48.32, 46.34, 33.65, 32.08, 31.78, 31.69, 29.25, 27.89, 26.89, 26.69, 22.70, 20.74, 14.19, 14.14 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{17}H_{33}NO_3$: 299.2460 $[M]^+$; found: 299.2462.

6-Bromo-5-oxo-hexanoic acid dihexylamide: 4-Dihexylcarbamoyl-butyric acid (1.61 g, 5.40 mmol) was dissolved in anhydrous dichloromethane (15 mL) at 0 °C and a catalytic amount of DMF was added. Oxalyl chloride was added (1.41 mL, 16.2 mmol) dropwise to this solution and the solution was then warmed to room temperature. After 1 h, the solvent and excess oxalyl chloride were removed under reduced pressure. The resulting oil was dissolved into anhydrous dichloromethane (10 mL) and added dropwise to a 0 °C solution of CH_2N_2 (16.20 mmol generated from Diazald/KOH) in diethyl ether (50 mL). This solution was stirred at 0 °C for 1.5 h, after which time, the reaction was quenched by the dropwise addition of 48% HBr (2.50 mL). The reaction mixture was diluted with dichloromethane (25 mL) and immediately washed with sat. $NaHCO_3$ (3×25 mL) and brine (2×25 mL) before being dried ($MgSO_4$), filtered, and concentrated. The crude yellow oil (0.780 g, 38%) was subsequently used in the next step without any further purification. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 4.33 (s, 2H), 2.45 (t, 2H, *J* = 8.8 Hz), 2.75 (t, 2H, *J* = 7.6 Hz), 2.41 (t, 2H, *J* = 7.6 Hz), 1.59 (m, 4H), 1.27 (br s, 12H), 0.86 ppm (m, 6H).

2-Amino-4-(3-dihexylcarbamoyl-propyl)imidazole-1-carboxylic acid *tert*-butyl ester: 6-Bromo-5-oxo-hexanoic acid dihexylamide (0.780 g, 2.07 mmol) and Boc-guanidine (0.988 g, 6.21 mmol) were dissolved in DMF (8 mL) and allowed to stir at room temperature. After 72 h, the DMF was removed under reduced pressure and the residue was taken up in ethyl acetate (100 mL) and washed with water (3×50 mL) and brine (50 mL) before being dried (Na_2SO_4), filtered, and evaporated to dryness. The resulting oil was purified by flash column chromatography (30–100% EtOAc/CH_2Cl_2) to give the title compound (0.643 g, 71%) as a white foam. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.47 (s, 1H), 6.41 (s, 2H), 3.42 (t, 4H, *J* = 7.6 Hz), 2.36 (m, 4H), 1.51 (br s, 13H), 1.25 (br s, 14H), 0.84 ppm (t, 6H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 183.99, 168.98, 149.95, 148.84, 136.98, 106.15, 103.11, 84.86, 84.06, 48.93, 30.75, 28.05, 27.71, 27.46, 25.35, 21.99, 20.89, 13.81 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{24}H_{44}N_4O_3$: 436.3413 $[M]^+$; found: 436.3412.

3-(2-Amino-1*H*-imidazol-4-yl)-*N*-(1-hexyl-heptyl)propionamide hydrochloride (34): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-[2-(1-hexyl-heptylcarbamoyl)ethyl]imidazole-1-carboxylic acid *tert*-butyl ester (0.130 g, 0.297 mmol) gave the title compound **34** (0.107 g, 97%) as an amber oil. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.92 (s, 1H), 11.62 (s, 1H), 7.65 (d, 1H, *J* = 8.7 Hz), 7.36 (s, 2H), 6.51 (s, 1H), 3.65 (m, 1H), 2.63 (t, 2H, *J* = 7.2 Hz), 2.36 (t, 2H, *J* = 7.2 Hz), 1.25 (m, 20H), 0.84 ppm (t, 6H, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 170.24, 146.57, 126.18, 108.50, 47.99, 34.51, 33.54, 31.17, 28.53, 25.39, 21.98, 20.37, 13.87 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{19}H_{36}N_4O$: 336.2889 $[M]^+$; found: 336.2894.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-(1-hexyl-heptyl)butyramide hydrochloride (35): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-[3-(1-hexyl-heptylcarbamoyl)propyl]imidazole-1-carboxylic acid *tert*-butyl ester (0.032 g, 0.071 mmol) gave the title compound **35** (0.027 g, 99%) as a tan-colored oil. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.99 (s, 1H), 11.57 (s, 1H), 7.52 (d, 1H, *J* = 8.7 Hz), 7.31 (s, 2H), 6.55 (s, 1H), 3.70 (m, 1H), 2.38 (t, 2H, *J* = 7.2 Hz), 2.08 (t, 2H, *J* = 7.2 Hz), 1.73 (m, 2H), 1.24 (m, 20H), 0.84 ppm (m, 6H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.00, 146.67, 126.46, 108.70, 47.80, 38.41, 34.59, 31.27, 31.07, 28.61, 25.50, 24.15, 23.60, 22.05, 13.96 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{20}H_{38}N_4O$: 350.3046 $[M]^+$; found: 350.3033.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*,*N*-dihexyl-butyramide hydrochloride (36): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-(3-dihexylcarbamoyl-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.562 g, 1.29 mmol) gave the title compound **36** (0.451 g, 94%) as a brown foam. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.21 (s, 1H), 11.67 (s, 1H), 7.39 (s, 2H), 6.50 (s, 1H), 3.43 (t, 4H, *J* = 7.5 Hz), 2.45 (m, 4H), 1.57 (m, 4H), 1.25 (br s, 14H), 0.85 ppm (t, 6H, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 146.78, 125.40, 108.98, 83.99, 49.07, 30.79, 28.11, 25.41, 24.33, 21.99, 20.63, 13.85 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{19}H_{36}N_4O$: 336.2889 $[M]^+$; found: 336.2897.

A native amide bond

5-Azido-1-bromo-pentan-2-one: 4-Azido-butyric acid[39] (2.15 g, 16.7 mmol) was dissolved in anhydrous dichloromethane (80 mL) at 0 °C and a catalytic amount of DMF was added. Oxalyl chloride (4.4 mL, 50 mmol) was added dropwise to this solution and the resulting mixture was then warmed to room temperature. After 1 h, the solvent and excess oxalyl chloride were removed under reduced pressure. The resulting oil was dissolved into anhydrous dichloromethane (10 mL) and added dropwise to a 0 °C solution of CH_2N_2 (50 mmol generated from Diazald/KOH) in diethyl ether (125 mL). This solution was stirred at 0 °C for 1.5 h, after which time, the reaction was quenched by the dropwise addition of 48% HBr (6.0 mL). The reaction mixture was diluted with dichloromethane (25 mL) and immediately washed with sat. $NaHCO_3$ (3×25 mL) and brine (2×25 mL) before being dried ($MgSO_4$), filtered, and concentrated. The amber oil obtained (3.10 g, 90%) upon concentration was pure and was used in the following steps without further purification. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 4.35 (s, 2H), 3.33 (t, 2H, *J* = 6.9 Hz), 2.66 (t, 2H, *J* = 7.2 Hz), 1.75 ppm (quint., 2H, *J* = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.33, 50.62, 36.63, 34.26, 23.25 ppm; HRMS (ESI): *m*/*z*: calcd for $C_5H_8BrN_3O$: 204.9851 $[M]^+$; found: 204.9850.

2-Amino-4-(3-azido-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (38): 5-Azido-1-bromo-pentan-2-one (0.870 g, 4.22 mmol) and Boc-guanidine (2.00 g, 12.7 mmol) were dissolved in DMF (15 mL) and allowed to stir at room temperature. After 24 h, the DMF was removed under reduced pressure and the residue was taken up in ethyl acetate (50 mL) and washed with water (3×25 mL) and brine (25 mL) before being dried (Na_2SO_4), filtered, and evaporated to dryness. The resulting oil was purified by flash column chromatography (10–100% EtOAc/hexanes) to give the title compound **38** (0.700 g, 63%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.53 (s, 1H), 5.98 (s, 2H), 3.30 (t, 2H, *J* = 6.9 Hz), 2.43 (t, 2H), *J* = 7.2 Hz), 1.87 ppm (quint., 2H, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 150.56, 149.56, 137.64, 107.09, 84.82, 50.89, 28.13, 27.79, 25.26 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{11}H_{18}N_6O_2$: 266.1491 $[M]^+$; found: 266.1499.

2-Amino-4-(3-heptanoylamino-propyl)imidazole-1-carboxylic acid *tert*-butyl ester: 2-Amino-4-(3-azido-propyl)imidazole-1-carboxylic acid *tert*-butyl ester **38** (0.100 g, 0.380 mmol) was added to a solution of anhydrous THF (4 mL) and 10% Pd/C (0.010 g). Air was removed from the system and the reaction was back flushed with hydrogen. This process was repeated three times before setting the reaction under a hydrogen balloon at atmospheric pressure and temperature for 8 h. After that time, heptanoic anhydride (0.104 mL, 0.390 mmol) was added dropwise to the reaction, which was then allowed to stir overnight. After this time, the reaction was filtered through a Celite pad and the filter cake was washed with THF (20 mL). The filtrate was concentrated under reduced pressure to afford the crude product, which was subsequently purified by flash column chromatography (0–10% MeOH/CH_2Cl_2) to give the title compound (0.093 g, 70%) as a white solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.76 (m, 1H), 6.52 (s, 1H), 6.38 (s, 2H), 3.02 (q, 2H, *J* = 6.9 Hz), 2.35 (t, 2H, *J* = 7.5 Hz), 2.03 (t, 2H, *J* = 7.5 Hz), 1.60 (quint., 2H, *J* = 7.2 Hz), 1.53 (s, 9H), 1.46 (m, 2H), 1.24 (m, 6H), 0.85 ppm (t, 3H, *J* = 6.6 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 171.89, 149.86, 148.90, 138.36, 105.71, 84.02, 38.10, 35.41, 30.98, 28.28, 27.87, 27.48, 25.27, 25.20, 21.97, 13.88 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{18}H_{32}N_4O_3$: 352.2474 $[M]^+$; found: 352.2488.

Heptanoic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (39): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-(3-heptanoylamino-propyl)imidazole-1-carboxylic acid *tert*-butyl ester (0.041 g, 0.120 mmol) gave the target compound **39** (0.033 g, 99%) as a tan-colored amorphous solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.08 (s, 1H), 11.61 (s, 1H), 7.91 (m, 1H), 7.33 (s, 2H), 6.57 (s, 1H), 3.03 (q, 2H, *J* = 6.8 Hz), 2.39 (t, 2H, *J* = 6.8 Hz), 2.05 (t, 2H, *J* = 6.4 Hz), 1.62 (t, 2H, *J* = 6.4 Hz), 1.47 (m, 2H), 1.23 (br s, 6H), 0.85 ppm (t, 3H, *J* = 6.4 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 172.21, 146.67, 126.34, 108.65, 37.49, 35.42, 30.99, 28.32, 27.84, 25.25, 21.98, 21.44, 13.90 ppm; HRMS (ESI): *m*/*z*: calcd for $C_{13}H_{24}N_4O$: 252.1950 $[M]^+$; found: 252.1957.

Examination of a triazole isostere

2-Amino-4-[3-(4-hexyl[1,2,3]triazol-1-yl)propyl]imidazole-1-carboxylic acid *tert*-butyl ester: Azide **38** (0.108 g, 0.406 mmol) was dissolved in a 1:1 mixture of ethanol (2 mL) and water (2 mL). Aodium-L-ascorbate (0.017 g, 0.081 mmol), $CuSO_4 \cdot 5H_2O$ (0.010 g, 0.041 mmol), and 1-octyne (0.054 g, 0.487 mmol) were added to this solution. The reaction was stirred at room temperature and monitored by TLC analysis. When completion of the reaction was evident, the ethanol was removed by rotary evaporation. The aqueous residue was diluted with water (50 mL) and EtOAc (100 mL). The organic layer was washed with sat. $NaHCO_3$ (3× 50 mL) and brine (50 mL), before being dried (Na_2SO_4), filtered, and evaporated to dryness. Purification of the crude product by flash column chromatography (0–10% $MeOH/CH_2Cl_2$) afforded the desired product (0.084 g, 55%) as an amber oil. ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta =$ 7.84 (s, 1H), 6.55 (s, 1H), 6.41 (s, 2H), 4.30 (t, 2H, $J = 6.9$ Hz), 2.58 (t, 2H, $J = 7.5$ Hz), 2.22 (t, 2H, $J = 6.9$ Hz), 2.02 (quint., 2H, $J = 6.9$ Hz), 1.55 (m, 11H), 1.27 (m, 6H), 0.85 ppm (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 149.93$, 148.86, 146.77, 137.46, 121.61, 106.02, 84.10, 48.68, 30.95, 28.89, 28.54, 28.19, 27.48, 24.99, 24.56, 21.96, 13.86 ppm; HRMS (ESI): m/z: calcd for $C_{19}H_{32}N_6O_2$: 376.2587 $[M]^+$; found: 376.2595.

4-[3-(4-Hexyl[1,2,3]triazol-1-yl)propyl]-1*H*-imidazol-2-ylamine hydrochloride (40): By using the same general procedure as that used for the synthesis of **8**, 2-amino-4-[3-(4-hexyl[1,2,3]triazol-1-yl)propyl]imidazole-1-carboxylic acid *tert*-butyl ester (0.054 g, 1.43 mmol) gave the title compound **40** (0.045 g, 100%) as an amber oil. ^1H NMR (300 MHz, [D$_6$]DMSO): $\delta = 12.12$ (s, 1H), 11.68 (s, 1H), 7.88 (s, 1H), 7.37 (s, 2H), 6.59 (s, 1H), 4.32 (t, 2H, $J = 6.9$ Hz), 2.59 (t, 2H, $J = 7.5$ Hz), 2.38 (t, 2H, $J = 7.2$ Hz), 2.06 (quint., 2H, $J = 7.5$ Hz), 1.57 (m, 2H), 1.27 (m, 6H), 0.85 ppm (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 146.83$, 146.78, 125.47, 121.83, 108.91, 48.33, 30.97, 28.90, 28.24, 28.01, 24.96, 21.99, 21.20, 13.91 ppm; HRMS (ESI): m/z: calcd for $C_{14}H_{24}N_6$: 276.2062 $[M]^+$; found: 276.2066.

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