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3-Oxoisoxazole-2(3*H*)-carboxamides and isoxazol-3-yl carbamates: Resistance-breaking acetylcholinesterase inhibitors targeting the malaria mosquito, *Anopheles gambiae*

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ABSTRACT

To identify potential selective and resistance-breaking mosquitocides against the African malaria vector *Anopheles gambiae*, we investigated the acetylcholinesterase (AChE) inhibitory and mosquitocidal properties of isoxazol-3-yl dimethylcarbamates (**15**), and the corresponding 3-oxoisoxazole-2(3*H*)-dimethylcarboxamide isomers (**14**). In both series, compounds were found with excellent contact toxicity to wild-type susceptible (G3) strain and multiply resistant (Akron) strain mosquitoes that carry the G119S resistance mutation of AChE. Compounds possessing good to excellent toxicity to Akron strain mosquitoes inhibit the G119S mutant of *An. gambiae* AChE (*Ag*AChE) with k_i values at least 10- to 600-fold higher than that of propoxur, a compound that does not kill Akron mosquitoes at the highest concentration tested. On average, inactivation of WT *Ag*AChE by dimethylcarboxamides **14** was 10–20 fold faster than that of the corresponding isoxazol-3-yl dimethylcarbamates **15**. X-ray crystallography of dimethylcarboxamide **14d** provided insight into that reactivity, a finding that may explain the inhibitory power of structurally-related inhibitors of hormone-sensitive lipase. Finally, human/*An. gambiae* AChE inhibition selectivities of these compounds were low, suggesting the need for additional structural modification.

1. Introduction

Malaria is one of the deadliest diseases known to mankind. Recent estimates suggest that approximately 207 million malaria cases and 625,000 deaths were attributed to this disease alone in 2012.[1] This problem is particularly severe in Sub-Saharan Africa where 90% of the cases occur. The much awaited malaria vaccine RTS,S/AS01 may help reduce the burden in malaria endemic regions. However, at present it has shown only partial efficacy in reducing malaria episodes in vaccinated children.[2] Until an effective vaccine is in place, other effective and economical interventions such as vector control are still needed. Insecticide treated nets (ITNs) have shown efficacy in reducing malaria transmission in the past in many malaria endemic regions.[3–5] At present, this method of intervention relies exclusively on pyrethroids, owing to their low mammalian toxicity and high insecticidal and repellant activity against the malaria vector, *Anopheles gambiae*. However, due to their widespread use, there has been an upsurge in mosquito populations resistant to this class of insecticides.[6] This development threatens to compromise the efficacy of ITNs, and has motivated the development of other classes of insecticides with different modes of action. Acetylcholinesterase (AChE) inhibitors appear promising in this regard, in view of their efficacy as an indoor wall application ('indoor residual spraying', IRS) against adult mosquitoes. However, none of the AChE inhibitors approved by the World Health Organization (WHO) for IRS (e.g., **1**, **2**) have been approved for ITNs, perhaps due to concerns of human toxicity. Thus, new safe and effective insecticides against the susceptible and resistant strains of *Anopheles gambiae* are needed for deployment on ITNs.

Our recently disclosed series of aryl methylcarbamates (e.g., **5**–**7**, Fig. 1) has shown high selectivity for *An. gambiae* AChE (*Ag*AChE) versus human AChE (*h*AChE).[7,8] These compounds

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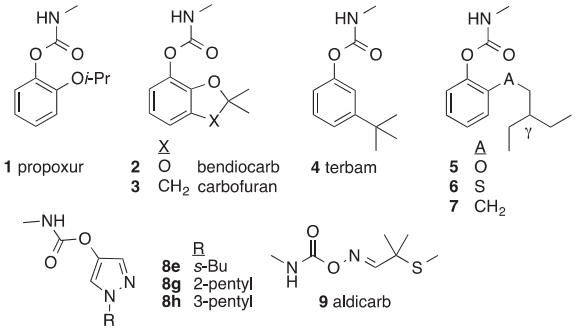


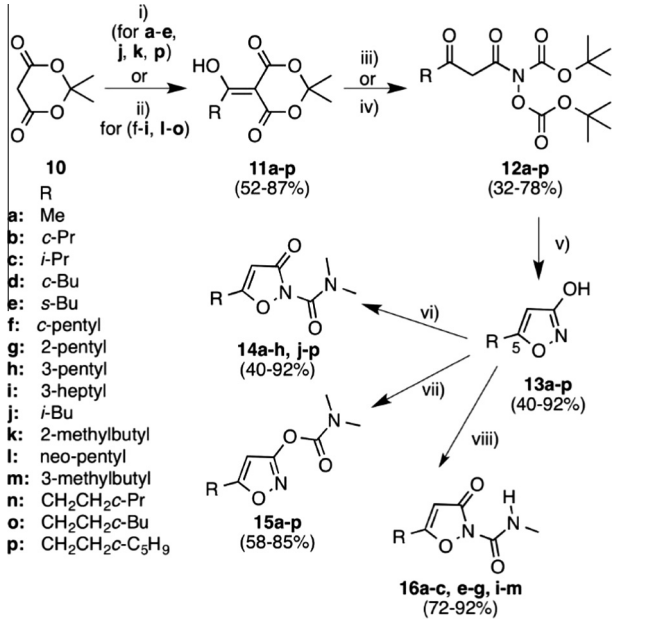
Figure 1. Structure of commercial carbamates (**1**–**4**, **9**), and previously reported aryl (**5**–**7**) and pyrazol-4-yl (**8e**, **8g**, **8h**) carbamates.

bearing a γ-branched substituent exhibit up to 500-fold selectivity for *Ag*AChE over *h*AChE, and are toxic to wild-type (WT) *An. gambiae* (G3 strain, MR4, CDC). This high selectivity must arise from amino acid substitutions near the active sites of *Ag*AChE and *h*AChE.[9] Although we cannot yet point to specific residue substitutions that **5**–**7** exploit to achieve selective inhibition, we have developed a ligand-based selectivity model.[8] However, these compounds, like aryl methylcarbamates **1**–**4**, are not appreciably toxic to Akron strain *An. gambiae* (MR4, CDC). Carbamate resistance of the Akron strain is known to arise from a G119S mutation in the oxyanion hole of AChE.[10,11] To compensate for reduced active site volume arising from this mutation, we prepared and assayed pyrazol-4-yl carbamates (e.g., **8e**, **8g**, **8h** Fig. 1) against susceptible (G3) and resistant (Akron) strain *An. gambiae*. Their excellent contact toxicity towards both strains and inhibition of wild-type (WT) and G119S *Ag*AChE suggests that a small core structure might be the key to combat carbamate resistance stemming from the G119S mutation.[12] The excellent Akron toxicity of commercial insecticide aldicarb (**9**) can also be explained by this steric argument.[12]

In this paper we disclose the synthesis, mosquitocidal and AChE inhibitory properties of carboxamides and carbamates based on an isoxazole core. The insecticidal properties of 3-oxoisoxazole-2(3*H*)-carboxamides have been documented in the patent literature,[13] but their contact toxicity to *An. gambiae* was not described. We will show that compounds from both structural classes can have high contact toxicity towards both WT and Akron resistant strain *An. gambiae*. The high insecticidal activity against resistant strain *An. gambiae* is particularly noteworthy.

2. Synthesis

Acylated Meldrum's acids **11a**–**p** were synthesized in moderate to high yield from **10** and the requisite acid chloride (or acid), using the published literature procedure (Scheme 1).[14] The R substituents were chosen to investigate the role of branching on inhibition selectivity, since they will reside at C5 of the isoxazole **13** (cf. **5**–**7** Fig. 1). Compounds **11b**–**i** feature α-branched alkyl groups, **11j**–**l** feature β-branched alkyl groups, and **11m**–**p** feature γ-branched alkyl groups. The subsequent thermolysis of **11** with *N*,*O*-bis-*t*-Boc hydroxylamine using the previously reported procedure[14] did not proceed smoothly in every case. In some instances (e.g., **12b**, **12c**, **12j**, **12k**), the product was obtained in low yield, whereas in other cases (**12m**–**o**), it could not be purified due to contamination with residual BocNHOBoc. To optimize the yield for these substrates, we took 1 equiv of the BocNHOBoc and 1.8 equiv of the compound **12l** and heated the reaction to 90 °C for 1.5–2 h. The reaction time for this step is crucial to avoid loss in yield due to incomplete conversion and competing side reactions. Using this modification, we could improve the yield of compound **12** for some

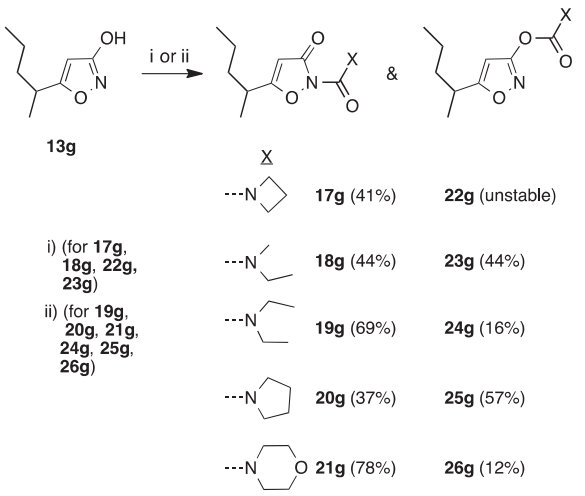


Scheme 1. Synthesis of 5-substituted 3-oxoisoxazole-2(3*H*)-carboxamides **14**, **16** and isoxazol-3-yl carbamates **15**. Reagents and conditions: (i) pyridine, DCM, 0 °C, 15 min; RC(O)Cl, 0 °C, 1.5 h; rt, 1.5 h; (ii) RCOOH, Et_3N, DECP, DMF, 0 °C, 30 min; rt, 16 h; (iii) NH(Boc)(OBoc), toluene, 90 °C, 1.5–2 h; (iv) NH(Boc)(OBoc), toluene, 65 °C, 16 h; (v) concd HCl, MeOH, 50 °C, 2.5 h; (vi) $ClC(O)NMe_2$, toluene, reflux, 16 h; (vii) KO*t*-Bu, THF, 0 °C, 15 min; $ClC(O)NMe_2$, rt, 16 h; (viii) KO*t*-Bu, THF, 0 °C, 15 min; ClC(O)NHMe, rt, 16 h.

substrates (e.g., **12a**, **12f**–**i**, **12l**) and the product could be isolated by column chromatography except in the case of **12d**, where we proceeded to the next step with the crude product. The cyclization of β-keto hydroxamic acid **12** proceeded in high yields even when the acid equivalents were reduced to half of that recommended in the literature.[14] The final carboxamide and carbamate products were obtained regioselectively through judicious use of two different protocols. The reaction of isoxazol-3-ol **13a**–**p** with dimethyl-carbamoyl chloride under neutral conditions gave 3-oxoisoxazole-2(3*H*)-carboxamides **14a**–**h**, **j**–**p** as the major products and isoxazol-3-yl dimethylcarbamate **15a**–**p** as the minor products (Scheme 1). Under neutral conditions, the more nucleophilic ring nitrogen attacks the carbonyl carbon of carbamoyl chloride forming dimethylcarboxamides as the major product. However, under basic conditions, **13a**–**p** gave isoxazol-3-yl dimethylcarbamate **15a**–**p** as the major product, with dimethyl-carboxamides **14a**–**p** formed in 5–17% yield (Scheme 1).

Unexpectedly, reaction of **13a**–**c**, **e**–**g**, and **i**–**m** with methylcarbamoyl chloride under basic conditions gave *N*-methyl-3-oxoisoxazole-2(3*H*)-carboxamide **16** with no trace of the corresponding methylcarbamates (Scheme 1). It is possible that hydrogen bonding between the carbamate NH and the isoxazol-3(*2H*)-one carbonyl in **16** renders the methylcarboxamides considerably more stable than the methylcarbamate (see Fig. 4A below). In that case, methylcarbamates initially formed under basic conditions could undergo *O*- to *N*-carbamoyl transfer to yield the observed methyl-carboxamides. In fact, we noted that methylcarboxamides such as **16** were themselves unstable in aqueous buffer (pH 7.7) over 30 min; it is therefore possible that any traces of methylcarbamate present in the reaction mixture would be even more unstable and decomposed during aqueous workup.

To probe the effect of the N-substituent on mosquitocidal and enzyme inhibition activities, various N-substituted derivatives of **13g** were synthesized as shown in Scheme 2. Compounds **17g**, **18g**, and **23g** were synthesized by reaction of **13g** with triphosgene and corresponding amine hydrogen chloride salt (**17g**) or amine



Scheme 2. Derivatization of isoxazol-3-ol **13g**. Reagents and conditions: (i) triphosgene, DCM, rt, 2 h; DIPEA, HX or HX·HCl, rt, 30 min; or (ii) ClC(O)X, toluene, reflux, 16 h.

(**18g**, **23g**). Compound **22g** was obtained in low yield from the same reaction mixture but appeared to be unstable, and hence was not assayed. Compounds **19g**, **20g**, and **21g** were synthesized by refluxing **13g** with corresponding carbamoyl chloride in toluene; the corresponding dimethylcarbamates **24g**, **25g**, and **26g**, were obtained in the same reactions. As expected, carboxamides predominated under neutral conditions, except in the case of the carbamoyl chloride derived from pyrrolidine, where the carbamate **25g** was obtained as the major product (Scheme 2).

2.1. Characterization of the 3-oxoisoxazole-2(3*H*)-carboxamides and isoxazol-3-yl carbamates

The final products were characterized and identified using ^{1}H and ^{13}C NMR, mass spectroscopy, and in two cases (**14d**, **16j**) X-ray diffraction. Assignments of the carboxamide and carbamate structures were made in the following way. As illustrated in Figure 2, dimethylcarboxamides (e.g., **14a**) show a broad singlet for the *N*-Me protons in the ^{1}H NMR spectrum, whereas dimethylcarbamates (e.g., **15a**) show two well-resolved singlets for these protons. The barrier to rotation of the C(O)–N bond in analogous urea-type dimethylcarboxamide analogues ranges from 8 to 13 kcal/mol,[15] whereas in dimethylcarbamates it is higher, typically ~16 kcal/mol.[16] These consistent differences in barriers to rotation presumably correspond to differences in the C(O)–N double bond character of ureas and carbamates. Similar behavior is seen in the ^{13}C NMR spectra of these compounds (Fig. 2B), where the *N*-Me carbons of **14a** appear as very low intensity, broadened signals, and the *N*-Me carbons of **15a** appear as two sharp resonances.

Another characteristic difference between 3-oxoisoxazole-2(3*H*)-carboxamides (both methyl- and dimethyl), and isoxazol-3-yl dimethylcarbamates was seen in ^{1}H NMR chemical shifts of H-4 (Fig. 3). This proton appeared between 5.27 ppm and 5.58 ppm for carboxamides **14** and **16**, whereas for isoxazol-3-yl dimethylcarbamates

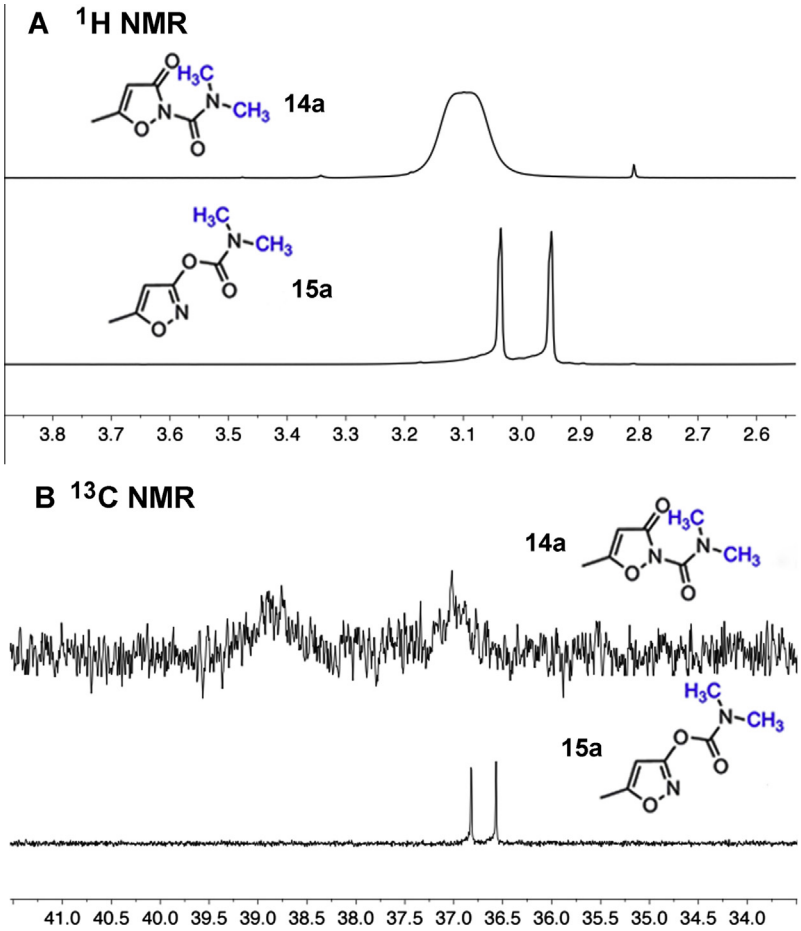


Figure 2. (A) The *N*-Me regions of the ^{1}H NMR spectra of 3-oxoisoxazole-2(3*H*)-carboxamide **14a** and isoxazol-3-yl carbamate **15a**. (B) The *N*-Me regions of the ^{13}C NMR spectra of these compounds.

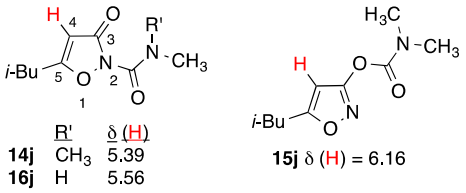


Figure 3. Characteristic chemical shifts of H-4 in 3-oxoisoxazole-2(3*H*)-carboxamides and isoxazol-3-yl dimethylcarbamates (ppm), as illustrated by **14j**, **16j**, and **15j**, respectively.

15 it appeared in the range 6.07–6.17 ppm (Fig. 3). To correlate this chemical shift difference to structure, we noted that the H-4 chemical shift of dimethylcarboxamide **14j** was similar to that of methylcarboxamide **16j**. The identity of **16j** as a methylcarboxamide was confirmed by X-ray crystallography (Fig. 4A).

The identity of **14d** (H-4 δ = 5.42 ppm) as a dimethyl-carboxamide was also confirmed by X-ray crystallography (Fig. 4B). The presence of the second methyl group (C9) eliminates the hydrogen bond seen in **16j**, and steric interaction of this group with O2 causes the exocyclic amide moiety to rotate out of the plane of the isoxazole ring. We will return to this point below in our discussion of inactivation rate constants k_i.

3. Tarsal contact toxicity of 3-oxoisoxazole-2(3*H*)-carboxamides and isoxazol-3-yl carbamates

Contact toxicity is a critical property for any insecticide that might be deployed on ITNs. Tarsal contact toxicity was determined towards G3 (WT, susceptible) and Akron (G119S, carbamate-resistant) strain *An. gambiae*, using a modification of the standard WHO tarsal contact toxicity protocol.[18] In the discussion below, contact toxicities will be classified as excellent toxicity (LC_{50} <100 μg/mL), good (LC_{50} = 100–199 μg/mL), moderate (LC_{50} = 200–399 μg/mL), and poor (LC_{50} ⩾400 μg/mL). As mentioned previously, commercial carbamates **1**–**4** bearing a phenyl core displayed excellent toxicities to the susceptible G3 strain of *An. gambiae* (LC_{50} = 16–39 μg/mL), but no measurable toxicity to resistant Akron strain (Table 1). In contrast pyrazol-4-yl methylcarbamate **8e** and aldicarb **9** had excellent toxicities to both strains[12] (Table 1). We attributed the low resistance ratios of these two compounds in part to their smaller core structures, and were thus interested to learn whether the isoxazol-3-yl core could also confer high toxicity to Akron strain *An. gambiae*. As noted above, isoxazol-3-yl methylcarbamates could not be isolated, and methylcarboxamides **16** proved unstable in aqueous buffer. Therefore our investigations focused on dimethylcarboxamides **14**, and dimethylcarbamates **15**. The carboxamide structure represents a significant departure from the structure of aryl and pyrazole carbamates that we previously studied. The possible effects of disubstitution on the carbamate nitrogen on contact toxicity were similarly unknown. We were pleasantly surprised to find that a number of dimethylcarboxamides were appreciably toxic to G3 and Akron strain *An. gambiae* (Table 1). Compound **14a** did not show any contact toxicity at the highest concentration tested (1000 μg/mL, Table 1), perhaps as a consequence of low lipophilicity. However, α-branched dimethylcarboxamides (**14b**–**h**) showed appreciable toxicities towards G3 and Akron strain (Table 2). Compound **14b** (R = *c*-Pr) showed moderate toxicity towards G3 strain, but was considerably less toxic against Akron strain. The open-chain analog

Table 1
Tarsal contact toxicity of control compounds (**1**–**4**, **8e**, **9**) and *N*,*N*-dialkyl-3-oxoisoxazole-2(3*H*)-carboxamides **14**, **17**–**21** to susceptible (G3) and resistant (Akron) strains of *An. gambiae*

Compound	R[a]	*An. gambiae* G3 LC_{50}[b] μg/mL (95% CI)	*An. gambiae* Akron LC_{50}[b] μg/mL (95% CI)	RR[c]
1[d]	NA	39 (32–45)	>5000[d]	>130
2[d]	NA	16 (14–17)	>5000	>310
3[d]	NA	16 (11–25)	>5000	>310
4[d]	NA	37 (14–16)	>5000	>130
8e[d]	NA	96 (89–104)	81 (78–89)	0.8
9[d]	NA	70 (66–74)	32 (30–35)	0.5
14a	Me	0% @ 1000 μg/mL	ND	–
14b	*c*-Pr	278 (225–345)	10% @ 1000 μg/mL	–
14c	*i*-Pr	316 (22–428)	182 (138–253)	0.6
14d	*c*-Bu	79 (57–116)	74 (55–105)	0.9
14e	*s*-Bu	63 (49–81)	129 (91–196)	2.0
14f	*c*-C_5H_{11}	105 (78–157)	118 (89–171)	1.1
14g	2-Pentyl	153 (109–211)	75 (53–111)	0.5
14h	3-Pentyl	38 (28–53)	40 (31–54)	1.1
14j	*i*-Bu	201 (145–284)	25% @ 200 μg/mL	–
14k	2-Methylbutyl	0% @ 1000 μg/mL	ND	
14l	*neo*-Pentyl	80% @ 1000 μg/mL	ND	–
14m	3-Methylbutyl	73 (58–93)	68 (54–87)	0.9
14n	CH_2CH_2-*c*-Pr	133 (71–226)	144 (82–229)	1.1
14o	CH_2CH_2-*c*-Bu	30% @ 1000 μg/mL	ND	–
14p	CH_2CH_2-*c*-C_5H_9	0% @ 1000 μg/mL	ND	–
17g	2-Pentyl	78% @ 1000 μg/mL	ND	–
18g	2-Pentyl	400 (320–483)	614 (537–684)	1.5
19g	2-Pentyl	15% @ 1000 μg/mL	ND	–
20g	2-Pentyl	77% @ 1000 μg/mL	ND	–
21g	2-Pentyl	45% @ 1000 μg/mL	ND	–

[a] NA signifies not applicable.
[b] Mosquitoes were exposed (1 h) to dried filter papers previously treated with ethanolic solutions of carbamates; mortality was recorded after 24 h. LC_{50} values derive from the concentrations of inhibitor used to treat the paper. ND designates 'not determined'; compounds that did not show significant toxicity to susceptible G3 strain (100% mortality at 1000 μg/mL) were generally not tested on the resistant Akron strain.
[c] Resistance ratio (RR), defined by LC_{50} (Akron)/LC_{50} (G3).
[d] Data reported previously.[12]

Table 2
Tarsal contact toxicity of isoxazol-3-yl dialkylcarbamates to G3 and Akron strain of *An. gambiae*

Compound	R	*An. gambiae* G3 LC_{50}[a] μg/mL (95% CI)	*An. gambiae* Akron LC_{50}[a] μg/mL (95% CI)	RR[b]
15a	Me	0% @1000 μg/mL	ND	–
15b	*c*-Pr	0% @1000 μg/mL	ND	–
15c	*i*-Pr	84 (30–124)	116 (79–151)	1.4
15d	*c*-Bu	349 (266–456)	392 (281–593)	1.1
15e	*s*-Bu	41 (28–58)	58 (42–92)	1.4
15f	*c*-Pentyl	60% @ 1000 μg/mL	ND	-
15g	2-Pentyl	234 (163–307)	296 (210–381)	1.3
15h	3-Pentyl	70% @ 1000 μg/mL	ND	–
15i	3-Heptyl	0% @ 1000 μg/mL	ND	–
15j	*i*-Bu	253 (184–367)	151 (112–211)	0.6
15k	2-Methylbutyl	30% @ 1000 μg/mL	ND	–
15l	*neo*-Pentyl	0% @ 1000 μg/mL	ND	–
15m	3-Methyl-butyl	415 (306–582)	429 (331–563)	1.0
15n	CH_2CH_2-*c*-Pr	191 (109–343)	198 (119–321)	1.0
15o	CH_2CH_2-*c*-Bu	40% @ 1000 μg/mL	ND	
15p	CH_2CH_2-*c*-C_5H_9	674 (575–715)	ND	–
23g	2-Pentyl	410 (304–590)	574 (513–633)	1.4
25g	2-Pentyl	0% @ 1000 μg/mL	ND	–
26g	2-Pentyl	10% @ 1000 μg/mL	ND	–

[a] Mosquitoes were exposed (1 h) to dried filter papers previously treated with ethanolic solutions of carbamates; mortality was recorded after 24 h. LC_{50} values derive from the concentrations of inhibitor used to treat the paper. ND designates 'not determined'; compounds that did not show significant toxicity to susceptible G3 strain (100% mortality at 1000 μg/mL) were generally not tested on the resistant Akron strain.
[b] Resistance ratio (RR), defined by LC_{50} (Akron)/LC_{50} (G3).

carboxamides **14c** (R = *i*-Pr), and **14g** (R = 2-pentyl) showed moderate and good G3 toxicity, respectively, and were even more toxic to the Akron strain. Compound **14d** (R = *c*-Bu) exhibited excellent toxicity towards both strains of *An. gambiae*. The compounds with highest G3 toxicity in the α-branched series were **14e** (R = *s*-Bu, LC_{50} = 63 μg/mL) and **14h** (R = 3-pentyl, LC_{50} = 38 μg/mL), and they showed good and excellent Akron toxicity, respectively. Amongst the β-branched dimethylcarboxamides explored (**14j–l**), only **14j** (R = *i*-Bu) showed moderate or better G3 toxicity, but Akron toxicity was lower. Among the γ-branched compounds explored (**14m–p**), the carboxamide **14m** (R = *i*-pentyl) was the most toxic and showed equivalent LC_{50} values towards G3 and Akron strain. A sharp decrease in G3 and Akron toxicity was observed from carboxamides **14n** (R = CH_2CH_2-*c*-Pr) to **14p** (R = CH_2CH_2-*c*-C_5H_9), concomitant with the increase in the size of the pendant carbocyclic ring. Finally, a range of compounds (**17g–21g**) were examined to assess the effect of varying the substitution on the exocyclic nitrogen. Only **18g** (ethylmethylcarboxamide) showed poor or better toxicity to G3 strain, and it was 2–3 fold less toxic than the dimethylcarboxamide **14g**. These data may suggest that the *N*-substituents of **17g–21g** are too big to inhibit *Ag*AChE well, and we address this point below. The low toxicity of azetidine carboxamide **17g** relative to dimethylcarboxamide **14g** may be due to instability of the azetidine ring, as discussed below.

Turning to the corresponding dimethylcarbamates, **15a** bearing a C5-methyl group was not toxic to G3 strain at the highest concentration tested (Table 2). In contrast α-branched analogs **15b–i** displayed G3 toxicities ranging from excellent (**15c**, **e**) to poor (**15b**, **f**, **h**, **i**, Table 2). Compounds **15c** (R = *i*-Pr) and **15e** (R = *s*-Bu) also demonstrated good and excellent toxicities towards Akron strain *An. gambiae*, respectively. One interesting trend is that open-chain analogs typically showed greater G3 toxicity than their cyclic analogs (cf. **15c** vs **15b**, **15e** vs **15d**, and **15g** vs **15f**). Finally **15i** (R = 3-heptyl) was not toxic to G3 strain at the highest concentration tested (1000 μg/mL).

Amongst the β-branched dimethylcarbamates examined (**15j–l**), only **15j** with an isobutyl side chain proved moderately toxic towards G3. Interestingly, this compound proved even more toxic to Akron strain than it was to G3 (cf. LC_{50} = 151 and 253 μg/mL, respectively). Amongst γ-branched compounds, unlike corresponding carboxamides, no clear trend was observed in G3 and Akron toxicity. The optimal LC_{50} was achieved for carbamates **15n** (R = CH_2CH_2-*c*-Pr, G3 LC_{50} = 191 μg/mL). **15m**, **15o**, and **15p** exhibited poor toxicity towards both strains of *An. gambiae*. Finally, as was seen for the carboxamides, variation of the N-substituent reduced toxicity. Only **23g** (ethylmethylcarbamate) showed moderate or better toxicity to G3 strain, but had reduced toxicity for Akron strain. Compound **25g** (pyrrolidinylcarbamate), and **26g** (morpholinocarbamate) did not show any toxicity to G3 strain *An. gambiae*.

Looking at both series, three conclusions can be drawn. First, for a given C5-substituent, there is no general trend for dimethylcarboxamide versus dimethylcarbamate toxicity. In some cases (**b**, **d**, **f**, **g**, **h**, **j**, **l**, **m**) the dimethylcarboxamide **14** is more toxic, and in others (**c**, **e**, **h**, **p**) the dimethylcarbamate **15** is more toxic. Second, among the exocyclic *N*-substituents explored, dimethyl proved optimum, though ethylmethyl analogs **18g** and **23g** did show significant toxicity. Third, both in the α- and γ-branched series, excellent G3 toxicity and low cross-resistance can be attained. The resistance ratios (RR) for **14c**, **14g** and **15j** are all less than 1.0, which signifies greater susceptibility of resistant strain *An. gambiae* towards these novel insecticides. This property would certainly be favorable for a new anticholinesterase-based mosquitocide.

4. Inhibition of WT and G119S *Ag*AChE by 3-oxoisoxazole-2(3*H*)-carboxamide and isoxazol-3-yl carbamates

Carbamates are pseudo-irreversible inhibitors of AChE; they inhibit the enzyme by carbamoylating the active site serine. We have previously used the Ellman Assay to monitor the time-dependent inhibitory activity of carbamates.[7,8,12,19] We employed the same method to determine the inhibition of AChE for our heterocyclic carbamates and carboxamides. Enzyme velocities (v/v_0) at a fixed inhibitor concentration were measured as a function of time *t*. Plots of $\ln(v/v_0)$ versus incubation time *t* were constructed and the slope provided the pseudo first-order rate constant k_{obs} (min^{-1}) for inactivation. For each inhibitor, k_{obs} values were determined at three or more inhibitor concentrations [*I*]. Finally, the linear fit slope of plots of k_{obs} versus [*I*] provided the second order rate constant k_i (mM^{-1} min^{-1}) for inactivation. Note that non-saturating [*I*] were chosen to give linear k_{obs} versus [*I*] plots.[7,8,12] The inhibition data and the sensitivity ratios for commercial carbamates are given in Table 3. As expected from their high toxicological resistance ratios (RR), the commercial aryl carbamates **1–4** inhibited the susceptible enzyme (WT AChE) much more rapidly than the resistant enzyme (G119S AChE), giving enzymatic sensitivity ratios (SR) greater than 3800-fold. In contrast, the SR values for pyrazol-4-yl methylcarbamate **8e** and aldicarb (**9**) were only 32- and 4.2-fold, which correlate well with their low toxicological resistance ratios (0.8 and 0.5, respectively).

On the basis of low toxicological resistance ratios seen for many isoxazol-3-yl dimethylcarbamates and dimethylcarboxamides (Tables 1 and 2), we predicted low SR values for these compounds. However, as will be seen below, this expectation was only partially realized. The k_i values for *N*,*N*-alkyl-3-oxoisoxazole-2(3*H*)-carboxamides are presented in Table 3. Compound **14a** (R = Me) showed very slow inactivation of *Ag*AChE-WT (0.46 mM^{-1} min^{-1}), which could be a consequence of high desolvation penalty (low lipophilicity). Dimethylcarboxamides bearing larger R groups at C5 showed much greater WT *Ag*AChE k_i values, ranging from 24.3 to

Table 3
Inactivation rate constants (k_i) of control compounds (**1**–**4**, **8e**, **9**) and carboxamides **14a**–**p**, **17g**–**21g** for r*Ag*AChE (WT and G119S) and r*h*AChE (h)

Compound	R[a]	WT k_i (mM^{-1} min^{-1})[b]	G119S k_i (mM^{-1} min^{-1})[b]	h k_i (mM^{-1} min^{-1})[b]	WT/G119S SR[c]
1[d]	NA	266 ± 9	<0.037 ± 0.007	17.0 ± 0.4	7200 ± 1400
2[d]	NA	839 ± 22	<0.055 ± 0.007	111 ± 5	15,000 ± 2000
3[d]	NA	2620 ± 150	<0.044 ± 0.020	428 ± 12	60,000 ± 27,000
4[d]	NA	1510 ± 100	0.40 ± 0.03	126 ± 3	3800 ± 400
8e[d]	NA	9140 ± 260	290 ± 7	805 ± 36	32 ± 1
9[d]	NA	13.3 ± 0.3	3.15 ± 0.08	6.5 ± 0.3	4.2 ± 0.1
14a	Me	0.46 ± 0.04	0.098 ± 0.029	1.22 ± 0.04	4.7 ± 1.4
14b	*c*-Pr	50.5 ± 1.4	0.20 ± 0.03	103 ± 5	250 ± 30
14c	*i*-Pr	500 ± 10	1.85 ± 0.09	561 ± 21	270 ± 10
14d	*c*-Bu	293 ± 9	0.62 ± 0.05	233 ± 13	480 ± 40
14e	*s*-Bu	2290 ± 80	10.6 ± 0.5	2170 ± 40	220 ± 10
14f	*c*-C_5H_{11}	1020 ± 50	2.58 ± 0.22	677 ± 26	400 ± 40
14g	2-Pentyl	5240 ± 140	14.7 ± 0.3	3110 ± 120	360 ± 10
14h	3-Pentyl	8530 ± 420	22.5 ± 1.1	1990 ± 70	380 ± 30
14j	*i*-Bu	252 ± 4	0.55 ± 0.02	30.9 ± 2.6	450 ± 20
14k	2-Methylbutyl	477 ± 7	0.99 ± 0.05	112 ± 5	480 ± 20
14l	*neo*-Pentyl	161 ± 12	0.37 ± 0.06	26.2 ± 1.1	440 ± 80
14m	3-Methylbutyl	266 ± 9	0.40 ± 0.12	80.9 ± 2.0	670 ± 200
14n	CH_2CH_2-*c*-Pr	420 ± 15	1.07 ± 0.06	144 ± 7	390 ± 30
14o	CH_2CH_2-*c*-Bu	141 ± 4	0.31 ± 0.09	74.5 ± 4.4	460 ± 130
14p	CH_2CH_2-*c*-C_5H_9	24.3 ± 0.1	0.41 ± 0.27	92.0 ± 4.0	60 ± 4.1
17g	2-Pentyl	2240 ± 80	36.7 ± 2.7	1860 ± 40	61 ± 5
18g	2-Pentyl	156 ± 6	3.84 ± 0.20	1080 ± 50	41 ± 3
19g	2-Pentyl	7.30 ± 0.09[c]	1.24 ± 0.05	180 ± 3 [c]	5.9 ± 0.3
20g	2-Pentyl	134 ± 8	1.90 ± 0.11	379 ± 24	71 ± 6
21g	2-Pentyl	39.0 ± 2.1	2.15 ± 0.27	26.0 ± 0.3	18 ± 3

[a] NA signifies not applicable.
[b] Measured at 23 ± 1 °C, pH 7.7, 0.1% (v/v) DMSO. Recombinant sources of *Ag*AChE are r*Ag*AChE-WT and r*Ag*AChE-G119S.
[c] Enzymatic sensitivity ratio is calculated as k_i(WT)/k_i(G119S). Standard error in the ratio is calculated according to a standard propagation of error formula.[20]
[d] Data reported previously.[12]

8,530 mM^{-1} min^{-1} (**14p** and **14h**, respectively). In general, the largest *Ag*AChE inactivation rate constants were realized with α-branched alkyl substitution, in particular **14e**–**h**. These dimethylcarboxamides also exhibited the largest G119S inactivation constants (2.58–22.5 mM^{-1} min^{-1}); the rate constants for **14g** and **14h** were approximately 5- and 7-fold higher than that of aldicarb (**9**). Although the WT/G119S enzymatic sensitivity ratios for these compounds are still very large (360- to 380-fold), they are 10-fold lower than that of **1**–**4**.

Comparison of cycloalkyl analogs **14b**, **d**, **f** with the corresponding open chain α-branched analogs **14c**, **e**, **g**, **h** shows that in each case, the open chain compounds offered significantly more rapid inactivation of WT or G119S *Ag*AChE than the cycloalkyl analogs (cf **14c** vs **14b**, **14e** vs **14d**, and **14g** or **14h** vs **14f**). It is possible that the flexibility of the side chain gives **14c**, **14e**, **14g**, and **14h** a better fit in the active site of the enzyme as compared to **14b**, **14d**, and **14f**. With regard to *Ag*AChE/*h*AChE selectivity, the α-branched carboxamides did not offer more than 4-fold selectivity. The three β-branched dimethylcarboxamides investigated (**14j**–**l**) exhibited reduced k_i values compared to those with α-branching. Unfortunately, selectivity for *Ag*AChE over *h*AChE within this series remained much lower than desired, reaching a maximum of 8-fold for **14j**. By analogy to compounds **5** to **7**, it was hoped that a γ-branched alkyl group would confer high inactivation selectivity. However within the γ-branched dimethylcarboxamide series (**14m**–**p**), the maximum *Ag*AChE inactivation selectivity achieved was 3-fold (**14n**). With regard to WT and G119S inactivation, an approximate two-fold increase in k_i was seen for **14n** (R = CH_2CH_2-*c*-Pr) compared to **14m** (R = CH_2CH_2-*i*-Pr). However, increasing size of the carbocycle in the series **14n**, **14o**, **14p** reduced both WT and G119S k_i values.

Variation of the exocyclic nitrogen substituents, while retaining a 2-pentyl group at C5, had dramatic effects on both WT and G119S *Ag*AChE k_i values. The relatively conservative replacement of the dimethylamino group of **14g** with an azetidine moiety (**17g**) reduced WT *Ag*AChE k_i value by 50%, but more than doubled the G119S *Ag*AChE k_i value. Replacement of one of the methyl groups of **14g** with an ethyl group (**18g**) reduced the WT *Ag*AChE k_i value 30-fold, and the G119S *Ag*AChE k_i value nearly 4-fold, suggesting steric inhibition of binding or carbamoylation in the acyl pocket of *Ag*AChE. Consistent with this hypothesis, pyrrolidino- and morpholino-carboxamides **20g** and **21g** offered more rapid inactivation than the diethylcarboxamide **19g**, but all three were much slower than dimethyl carboxamide **14g**.

Turning to the k_i values of the dimethylcarbamates, compound **15a** (like its carboxamide counterpart **14a**) exhibited extremely slow inactivation of all three enzymes; again low lipophilicity may play a role (Table 4). Among α-, β-, and γ-branched dimethylcarbamates, the highest WT and G119S *Ag*AChE values were seen for the α-branched compounds (e.g., **15e**–**h**), as was the case for dimethylcarboxamides **14** (Table 3). The highest G119S *Ag*AChE k_i values in the dimethylcarbamate series are in the range of the dimethylcarboxamides; in particular **15h**, **15e**, and **15g** have G119S k_i values ranging from 3- to 10-fold of that of aldicarb (**9**). However, the α-branched dimethylcarbamates **15e**–**h** exhibited at least 10-fold slower inactivation of WT *Ag*AChE than the corresponding dimethylcarboxamides **14e**–**h**. Consequently, the enzymatic sensitivity ratios of the dimethylcarbamates are roughly 10-fold lower than that of the dimethylcarboxamides (cf. SR values in Tables 3 and 4). As was seen for dimethylcarboxamides, WT and G119S *Ag*AChE k_i values for open chain α-branched dimethylcarbamates were higher than those of their cycloalkyl homologs (cf. **15c** vs **15b**, **15e** vs **15d**, **15h**, **g** vs **15f**).

Among the β-branched dimethylcarbamates, the bulky neopentyl compound **15l** was much less inhibitory at all three enzymes than the isobutyl (**15j**) and 2-methylbutyl (**15k**) compounds. The γ-branched dimethylcarbamates **15m**–**p** were again examined in the hope that they (like **5**–**7**) would exhibit good WT *Ag*/*h*

Table 4
Inactivation rate constants (k_i) of isoxazol-3-yl dialkylcarbamates for r*Ag*AChE (WT and G119S), and r*h*AChE (h)

Compound	R	WT k_i (mM^{-1} min^{-1})[a]	G119S k_i (mM^{-1} min^{-1})[a]	h k_i (mM^{-1} min^{-1})[a]	WT/G119S SR[b]
15a	Me	0.0323 ± 0.0082[c]	0.0224 ± 0.0125	0.118 ± 0.019	1.4 ± 0.9
15b	*c*-Pr	3.02 ± 0.13	0.30 ± 0.03	2.02 ± 0.27	10 ± 1
15c	*i*-Pr	7.71 ± 0.20	0.50 ± 0.04	4.46 ± 0.12	15 ± 1
15d	*c*-Bu	6.84 ± 0.20	0.44 ± 0.04	1.86 ± 0.10	16 ± 2
15e	*s*-Bu	323 ± 6	20.4 ± 0.6	60.1 ± 1.8	16 ± 1
15f	*c*-Pentyl	64.7 ± 2.7	1.94 ± 0.04	4.62 ± 0.07	33 ± 2
15g	2-Pentyl	416 ± 7	30.6 ± 0.5	50.9 ± 1.0	14 ± 1
15h	3-Pentyl	221 ± 7	10.5 ± 0.7	9.04 ± 0.31	21 ± 2
15i	3-Heptyl	2.85 ± 0.11	0.26 ± 0.01	2.13 ± 0.09	11 ± 1
15j	*i*-Bu	10.6 ± 0.5	0.87 ± 0.06	1.59 ± 0.08	12 ± 1
15k	2-Methylbutyl	7.64 ± 0.21	0.50 ± 0.05	1.70 ± 0.09	15 ± 2
15l	*neo*-Pentyl	0.12 ± 0.03	0.05 ± 0.01	0.44 ± 0.04	2.4 ± 0.7
15m	3-Methylbutyl	3.12 ± 0.16	0.37 ± 0.03	1.91 ± 0.04	8.3 ± 0.8
15n	CH_2CH_2-*c*-Pr	23.5 ± 1.1	2.01 ± 0.05	8.26 ± 0.26	12 ± 1
15o	CH_2CH_2-*c*-Bu	5.08 ± 0.23	0.62 ± 0.03	7.24 ± 0.90	8.2 ± 0.6
15p	CH_2CH_2-*c*-C_5H_9	0.60 ± 0.05	0.12 ± 0.03	13.1 ± 0.4	5.2 ± 1.2
23g	2-Pentyl	24.5 ± 0.5	3.37 ± 0.20	9.14 ± 1.97	7.3 ± 0.5
25g	2-Pentyl	12.4 ± 0.1[c]	2.24 ± 0.08[c]	1.62 ± 0.07[c]	5.5 ± 0.2
26g	2-Pentyl	18.6 ± 0.3[c]	2.30 ± 0.10[c]	2.68 ± 0.06[c]	8.1 ± 0.4

[a] Measured at 23 ± 1 °C, pH 7.7, 0.1% (v/v) DMSO. Recombinant sources of *Ag*AChE are rAgAChE-WT and rAgAChE-G119S.
[b] Enzymatic sensitivity ratio (SR) is calculated as k_i(WT)/k_i(G119S). Standard error in the ratio is calculated according to a standard propagation of error formula.[20]
[c] k_i values extrapolated from single point incubation at various inhibitor concentration, for example, t = 10 min.

selectivity. However, as can be seen by inspection of the *Ag*AChE and *h*AChE k_i values in Table 4, the highest selectivity (24-fold) was obtained for the α-branched compound **15h**. Within the γ-branched series **15m**–**p**, the highest values of WT and G119S *Ag*AChE k_i were again seen for R = CH_2CH_2-*c*-Pr (**15n**). Finally, with regard to variation of the exocyclic *N*-substituents, increasing steric bulk reduced k_i values at all three enzymes (cf **23g**, **25g**, **26g** vs **15g**) again suggesting steric crowding in the acyl pocket of AChE.

5. Discussion

To gain insight into the unexpectedly high inactivation rates constants (k_i) of the dimethylcarboxamides relative to the dimethylcarbamates, we modeled inhibitor **14c** in the active site of mouse AChE, and compared it to the experimental X-ray structure of mouse AChE (*m*AChE) covalently bound by **27**, a potent trifluoromethylketone (TFK) inhibitor (Fig. 5).[21]

Flexible ligand docking of the dimethylcarboxamide tetrahedral covalent intermediate adduct derived from **14c** and mAChE was performed in ICM using default settings for 'covalent' docking mode (ICM-Docking module, Molsoft).[24,25] The choice of *m*AChE was motivated by three factors: (1) availability of a high-resolution crystal structure of its complex with **27** (PDB ID 2H9Y);[26] (2) high sequence identity (88%) of *m*AChE and *h*AChE;[27] and (3) our observation of fast inactivation of *h*AChE by the dimethylcarboxamides (cf. Table 3). The choice to model the tetrahedral intermediate rather than the carbamoylated AChE reflects our assumption that formation of the tetrahedral intermediate is likely the rate-limiting

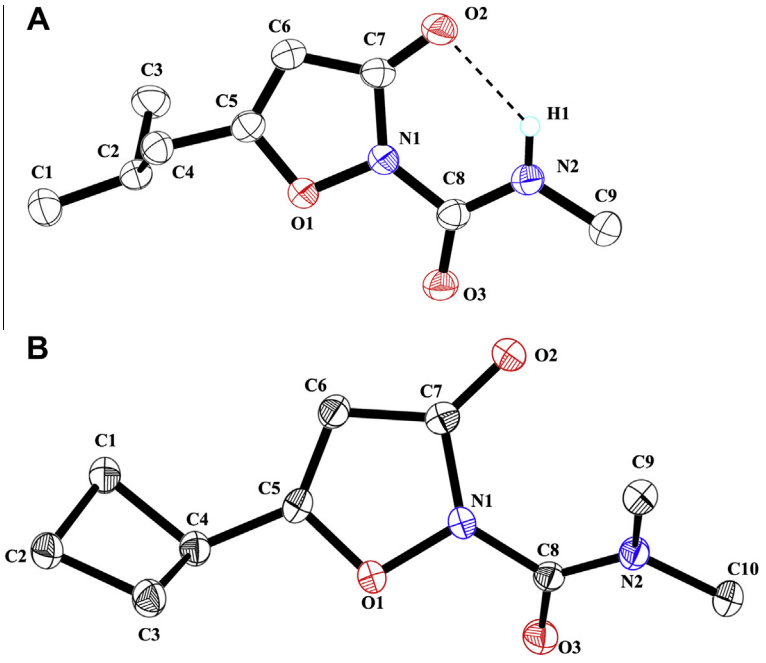


Figure 4. (A) Anisotropic displacement ellipsoid drawings (50%) of X-ray structure of compound **16j**. Hydrogen atoms were omitted for clarity, except at the exocyclic *N*. A predicted hydrogen bond is shown with a dotted black line (H1–O2 distance 2.11 Å). (B) Anisotropic displacement ellipsoid drawings (50%) of X-ray structure of compound **14d**. These structures were deposited at the Cambridge Structural Database (CCDC-1034990 and CCDC-1034991) and were visualized using OLEX2.[17]

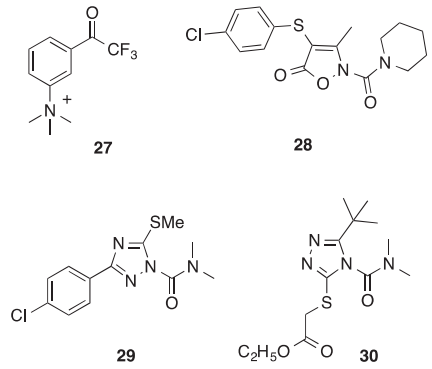


Figure 5. Serine hydrolase inhibitors related to dimethylcarboxamides **14**.[13,22,23]

step of enzyme inactivation, which then leads quickly to the carbamoylated enzyme. Figure 6 shows the tetrahedral adduct formed after the attack of active site serine (S203) on the carbonyl of TFK **27** (Fig. 6A) and **14c** (Fig. 6B). In both cases, the oxyanion formed is stabilized through hydrogen bonding with oxyanion hole residues G121, G122 and A204. Note that the corresponding active site serine and oxyanion residues in *Ag*AChE are S199, G118, G119, and A200, respectively, (see Supplementary data Fig. S1). The overlay of the two structures in Figure 6C highlights the similar pose of the two molecules in the active site of AChE: the NMe_2 group of **14c** overlays the trifluoromethyl group of **27** in the acylpocket. It is well known that the CF_3 group is similar in volume to an isopropyl group;[28] as Figure 6C illustrates, it is also similar in size to NMe_2. Lastly, the C5-*i*-Pr substituent of **14c** occupies the same locus as the trimethylammonium group of **27**, in the choline-binding site. It is possible that the C5-alkyl substituents of **14c** and analogs could have van der Waals interactions with W86, in place of the cation–π interaction experienced by **27**. Given these similarities in the predicted binding pose of **14c** and **27** to mouse AChE, the high k_i values seen at *h*AChE (and by extension *Ag*AChE) are more readily understood.

As mentioned earlier, the X-ray crystal structure of **14d** (Fig. 4B) also provides some insight into the reactivity of the dimethylcarboxamides. The extent of resonance between the endocyclic N (N1) and the amide carbonyl carbon (C8) appears to be low based on the out-of-plane twisting of the dimethylcarbamoyl group. The magnitude of amide twist can be expressed as the τ value[29,30] along a given N–C bond. As expected, the endocyclic amide bond N1–C7 has a low value (−12.6°), as does the dimethylamino amide bond N2–C8 (8.6°). However, the bond from N1 to the reactive carbonyl carbon C8 has a much larger twist, with a τ value of 47.5°. As mentioned earlier, this twist appears to be caused by steric interaction of C9 and O2, and the ensuing lack of resonance donation of N1 into the C8 carbonyl should serve to increase the electrophilicity of the dimethylcarboxamides. We note that N1 in **14d** is also quite pyramidalized (the sum of angles at N1 is only 346°). Additional details on this structure, and for that of **16j** are provided in the Supplementary material. Close analogs of these carboxamides have been cited in the literature as effective serine hydrolase inhibitors. Isoxazolonyl carboxamide[22] **28** and 1,2,4-triazole carboxamide[23] **29** are potent hormone-sensitive lipase (HSL) inhibitors (Fig. 5). Like vertebrate AChE, HSL belongs to the family of serine hydrolases with a α/β hydrolase fold.[31] Since they originated from the same ancestor, the catalytic residues are preserved in these enzymes. Triazamate **30**, a carbamoyl triazole AChE inhibitor aphicide, has been approved for plant protection in many European countries.[32] Based on the structural similarity of these compounds to **14d**, it seems likely that the serine hydrolase inhibitory power of **28**–**30** derives in part from a similar out-of-plane twist of the exocyclic amide moiety.

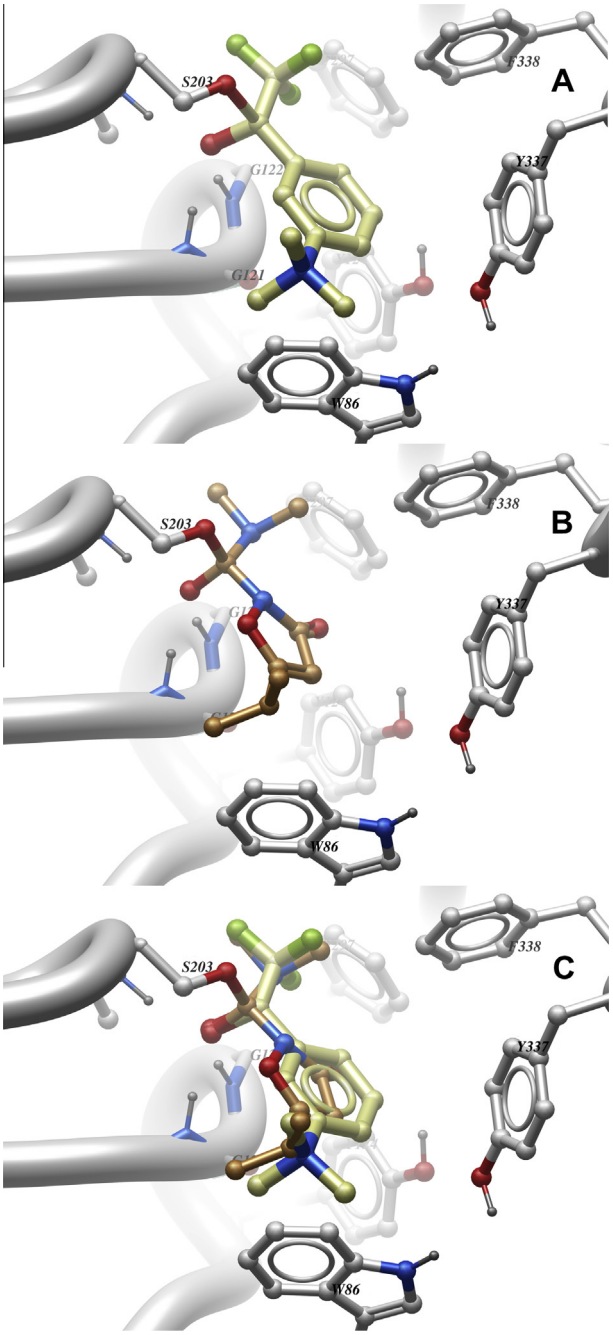


Figure 6. Experimental and modeled tetrahedral adducts of mouse AChE active site serine S203 with electrophilic carbonyl derivatives. (A) X-ray structure of the tetrahedral adduct of **27** with S203 (PDB ID 2H9Y).[26] (B) Computational modeling of the tetrahedral adduct of dimethylcarboxamide **14c** with S203 of mouse AChE. (C) Overlay of structures (A) and (B). In both cases the backbone NH moieties of oxyanion hole residues G121, G122 and A204 are within hydrogen bonding range of the anionic oxygen. Image created with Molsoft Browser Pro version 3.7-a.

Finally, it would seem obvious to expect that G3 toxicity and WT *Ag*AChE k_i values would be highly correlated. However, as we have shown for aryl methylcarbamates, ADME can be very influential,[7] and no general correlation is seen between G3 LC_{50} and *Ag*AChE k_i values in either the isoxazole carboxamide or carbamate series. For example, the α-branched compounds **14h** and **15e** have almost equal toxicities towards G3 *An. gambiae* (38 and 41 μg/mL, respectively). Despite the similar toxicities, **15e** is 26-fold less inhibitory than **14h** (323 and 8530 $mM^{-1}\,min^{-1}$, respectively). Similarly, **14j** and **14m** have similar *Ag*AChE (WT) k_i values (252

and 266 mM^{-1} min^{-1}, respectively), but the G3 LC_{50} value of **14j** is 2.4 fold higher than of **14m** (201 and 73 μg/mL, respectively). Further, despite the fact that these two compounds have similar G119S *Ag*AChE k_i values, **14m** is considerably more toxic to Akron strain than **14j**. In all these cases, differential pharmacokinetics and metabolism may be dominant factors in toxicity. Nevertheless, we can offer the following observations. Firstly, C5-methyl dimethylcarboxamide **14a** and dimethylcarbamate **15a** had very poor toxicity and very slow enzyme inactivation rate constants, likely as a consequence of low lipophilicity. The calculated *C*Log*P* values for these compounds are 0.70 and 0.20, as compared to 2.2 and 1.7 for the *s*-Bu substituted compounds **14e** and **15e**.[33] It is also possible that if the C5-substituent is insufficiently large, the carbamoyl group will not be positioned properly to react with the active site serine. Secondly, as previously mentioned, open chain α-branched dimethylcarbamates were more toxic to G3 strain than the corresponding cycloalkyl analogs (cf **15c** vs **15b**, **15e** vs **15d** and **15g** vs **15f**), and this trend correlates well with differences in enzyme inactivation k_i values. Third, increasing the size of the exocyclic *N*-substituent reduced toxicity in both dimethylcarboxamide (**14g** vs **17**–**21g**) and dimethylcarbamate (**15g** vs **23**, **25g**, **26g**), and decreased enzyme inactivation k_i values accompany these decreased toxicities. Fourth, for those compounds having excellent (**14d**, **14g**, **14h**, **14m**, and **15e**) and good (**14c**, **14e**, **14f**, **14n**, **15c**, **15j**, and **15n**) toxicity to Akron strain *An. gambiae*, we examined the corresponding G119S *Ag*AChE k_i values. All these compound give measurable k_i values at the G119S enzyme, ranging from 13% (**14m**) to 700% (**14h**) of the k_i value of aldicarb (**9**). The fact that Akron/G3 toxicological resistance ratios (RR) for these compounds are much smaller than the WT/G119S enzyme sensitivity ratios (SR) may reflect enhanced bioactivation or compromised detoxification in Akron, relative to G3. Nevertheless it seems likely that the Akron-toxic properties of these compounds indeed stem from engagement of the G119S *Ag*AChE target. This clearly advantageous property would be even more valuable, if it could be achieved in compound class that offers excellent selectivity against inhibition of *h*AChE. Work to achieve this goal is in progress.

6. Experimental

6.1. Chemistry

NMR spectra were obtained on JEOL Eclipse-plus 500 MHz spectrometer at 500 (^{1}H) and 126 (^{13}C) MHz or Unity-plus 400 at 400 (^{1}H) and 101 (^{13}C) MHz. The chemical shifts are reported in δ (ppm), and coupling constants are given in Hz. High-resolution ESI mass spectra were obtained on an Agilent 6220 accurate mass TOF LC/MS. X-ray data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlisPro. The structure was solved using SHELXS-2013 and refined using SHELXL-2013 via OLEX2. THF for moisture sensitive reactions was distilled from sodium-benzophenone. Other dry solvents were purchased from EMD Millipore and were used without any further purification. Column chromatography was performed using Silica gel (ZEOprep 60 ECO 40–63 μ) was purchased from AIC. Reagents were purchased mainly from Sigma Aldrich and were used as received.

6.2. General procedure for the synthesis of acyl Meldrum's acids

Method A: Adapted from the procedure of Sørensen et al.[14] To a solution of Meldrum's acid (1 equiv) in dichloromethane at 0 °C was added pyridine (2 equiv) drop wise, and the resulting solution was stirred for 15 min. The corresponding acid chloride (1 equiv) was added to this reaction mixture. Thereafter, the reaction was stirred for 1.5 h at 0 °C, and for an additional 1.5 h at room temperature. The reaction was quenched with 2 M hydrochloric acid, and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography using a gradient from 5% to 10% ethyl acetate in hexane, 1% acetic acid to yield acyl Meldrum's acids (**11a**–**e**, **j**, **l**, **p**).

Method B: Adapted from the procedure of Sørensen et al..[14] Meldrum's acid (1 equiv) and corresponding acid (1 equiv) were dissolved in DMF, and cooled to 0 °C. To this solution was added diethyl cyanophosphonate (1.1 equiv) and triethylamine (3.1 equiv) drop wise. The mixture was allowed to stir at 0 °C for 30 min followed by 16 h at room temperature. The reaction was quenched with 2 M hydrochloric acid and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, and dried over sodium sulfate. The solution was filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography using a gradient from 5% to 10% ethyl acetate in hexane, 1% acetic acid to yield acyl Meldrum's acids (**11f**–**i**, **k**, **m**, **n**, **o**).

6.2.1. 5-(1-Hydroxyethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11a)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (500 mg, 3.47 mmol), acetyl chloride (0.25 mL, 3.47 mmol), pyridine (0.71 mL, 6.94 mmol), and CH_2Cl_2 (4.2 mL). Aqueous workup, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11a** as a white solid (536 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.12 (s, 1H), 2.67 (s, 3H), 1.73 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.8, 170.3, 160.6, 105.1, 92.0, 27.0, 23.7.

6.2.2. 5-(Cyclopropyl(hydroxy)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11b)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (500 mg, 3.46 mmol), cyclopropyl chloride (0.32 mL, 3.46 mmol), pyridine (0.56 mL, 6.93 mmol), and CH_2Cl_2 (5.0 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11b** as a yellowish oil (462 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.41 (s, 1H), 3.54–3.44 (m, 1H), 1.74 (s, 6H), 1.49–1.41 (m, 2H), 1.32–1.20 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.1, 170.7, 161.4, 104.8, 91.2, 26.9, 15.7, 14.3.

6.2.3. 5-(1-Hydroxy-2-methylpropylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11c)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (2.00 g, 13.9 mmol), isobutyryl chloride (1.46 mL, 13.9 mmol), pyridine (2.24 mL, 27.7 mmol), and CH_2Cl_2 (17.0 mL). Aqueous workup, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11c** as a yellowish oil (1.77 g, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.53 (s, 1H), 4.08 (hept, J = 6.8 Hz, 1H), 1.73 (s, 6H), 1.23 (d, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.6, 171.1, 160.1, 104.8, 90.3, 33.2, 26.9, 19.2.

6.2.4. 5-(Cyclobutyl(hydroxy)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11d)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (500 mg, 3.47 mmol), cyclobutane carbonyl chloride (0.40 mL, 3.47 mmol), pyridine (0.71 mL, 6.94 mmol), and CH_2Cl_2 (4.2 mL). Aqueous work up and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11d** as a yellowish oil (604 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.53 (s, 1H), 4.47 (quintet, J = 8.5 Hz, 1H), 2.42–2.27 (m, 4H), 2.12–2.01 (m, 1H), 1.96–1.86 (m, 1H), 1.72 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.3, 170.8, 160.0, 104.8, 89.8, 39.3, 26.8, 25.4, 17.9. HRMS (ESI) calcd for $C_{11}H_{14}O_5$ $[M-H]^-$ 225.0768, found 225.0759.

6.2.5. 5-(1-Hydroxy-2-methylbutylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11e)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (3.00 g, 20.8 mmol), 2-methyl butyryl chloride (2.58 mL, 20.8 mmol), pyridine (3.36 mL, 41.6 mmol), and CH_2Cl_2 (25.0 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11e** as a yellowish oil (2.44 g, 52%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.48 (s, 1H), 3.97 (hextet, J = 6.9 Hz, 1H), 1.81–1.74 (m, 1H), 1.73 (s, 6H), 1.54 (doublet of quintet, J = 14.6, 7.3 Hz, 1H), 1.21 (d, J = 6.8 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.2, 170.9, 160.3, 104.8, 91.3, 39.5, 27.3, 27.0, 26.8, 17.1, 11.9. HRMS (ESI) calcd for $C_{11}H_{16}O_5$ $[M+Na]^+$ 251.0895, found 251.1618.

6.2.6. 5-(Cyclopentyl(hydroxy)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11f)

Prepared using the general procedure above (*Method B*) from Meldrum's acid (500 mg, 3.47 mmol), cyclopentanoic acid (0.38 mL, 3.47 mmol), diethyl cyanophosphonate (0.58 mL, 3.82 mmol), triethylamine (1.52 mL, 10.7 mmol), and DMF (7.2 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11f** as an off white solid (721 mg, 86%); mp 68.8–70.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.51 (s, 1H), 4.24–4.17 (m, 1H), 2.08–1.98 (m, 2H), 1.88–1.75 (m, 5H), 1.73 (s, 7H), 1.71–1.66 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.6, 171.0, 160.4, 104.8, 90.8, 43.9, 31.1, 26.9, 26.6. HRMS (ESI) calcd for $C_{12}H_{16}O_5$ $[M-H]^-$ 239.0925, found 239.0935.

6.2.7. 5-(1-Hydroxy-2-methylpentylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11g)

Prepared using the general procedure above (*Method B*) from Meldrum's acid (3.00 g, 20.8 mmol), 2-methyl valeric acid (2.60 mL, 20.8 mmol), diethyl cyanophosphonate (3.47 mL, 22.9 mmol), triethylamine (8.99 mL, 64.6 mmol), and DMF (25.0 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11g** as a yellowish oil (3.96 g, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.46 (s, 1H), 4.05 (hextet, J = 6.8 Hz, 1H), 1.71 (s, 6H), 1.50–1.24 (m, 4H), 1.20 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.2, 170.9, 160.2, 104.7, 91.1, 37.8, 36.2, 26.9, 26.7, 20.6, 17.5, 14.1. HRMS (ESI) calcd for $C_{12}H_{18}O_5$ $[M-H]^-$ 241.1081, found 241.1091.

6.2.8. 5-(2-Ethyl-1-hydroxybutylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11h)

Prepared using the general procedure above (*Method B*) from Meldrum's acid (2.00 g, 13.9 mmol), 2-ethylbutyric acid (1.75 mL, 13.9 mmol), diethyl cyanophosphonate (2.32 mL, 15.3 mmol), triethylamine (5.99 mL, 43.0 mmol), and DMF (28.8 mL). Aqueous workup and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11h** as a yellowish oil (1.65 g, 49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.43 (s, 1H), 4.02–3.93 (m, 1H), 1.73 (s, 6H), 1.77–1.68 (m, 2H), 1.68–1.58 (m, 2H), 0.92 (t, J = 7.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.6, 170.7, 160.5, 104.7, 93.0, 46.1, 26.9, 25.9, 11.8. HRMS (ESI) calcd for $C_{12}H_{18}O_5$ $[M-H]^-$ 242.1116, found 242.1096.

6.2.9. 5-(2-Ethyl-1-hydroxyhexylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11i)

Prepared using the general procedure above (*Method B*) from Meldrum's acid (2.00 g, 13.9 mmol), 2-ethylhexanoic acid (2.21 mL, 13.9 mmol), diethyl cyanophosphonate (2.32 mL, 15.3 mmol), triethylamine (5.99 mL, 43.0 mmol), and DMF (28.8 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11i** as a yellowish oil (2.82 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.42 (s, 1H), 4.04 (tt, J = 8.7, 5.6 Hz, 1H), 1.73 (s, 6H), 1.72–1.52 (m, 4H), 1.34–1.18 (m, 4H), 0.91 (t, J = 7.5 Hz, 3H), 0.87 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.8, 170.8, 160.5, 104.7, 92.8, 44.6, 32.5, 29.6, 26.9, 26.9, 26.4, 22.9, 14.0, 11.8. HRMS (ESI) calcd for $C_{14}H_{22}O_5$ $[M-H]^-$ 269.1394, found 269.1421.

6.2.10. 5-(1-Hydroxy-3-methylbutylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11j)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (300 mg, 2.08 mmol), isovaleryl chloride (0.25 mL, 2.08 mmol), pyridine (0.34 mL, 4.16 mmol), and CH_2Cl_2 (2.5 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11j** as a yellowish oil (274 mg, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.30 (s, 1H), 2.98 (d, J = 7.1 Hz, 2H), 2.26–2.14 (m, 1H), 1.73 (s, 6H), 1.01 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.7, 170.7, 160.4, 104.8, 92.0, 44.0, 27.5, 27.0, 22.7. HRMS (ESI) calcd for $C_{11}H_{16}O_5$ $[M-H]^-$ 227.0925, found 227.0939.

6.2.11. 5-(1-Hydroxy-3-methylpentylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11k)

Prepared using the general procedure above (*Method B*) from Meldrum's acid (300 mg, 2.08 mmol), 3-methylvaleric acid (0.26 mL, 2.08 mmol), diethyl cyanophosphonate (0.35 mL, 2.29 mmol), triethylamine (0.86 mL, 6.45 mmol), and DMF (4.3 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11k** as a yellowish oil (393 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.31 (s, 1H), 3.08 (dd, J = 13.4, 7.2 Hz, 1H), 2.91 (dd, J = 13.4, 7.2 Hz, 1H), 2.04–1.93 (m, 1H), 1.72 (s, 6H), 1.44 (doublet of quintet, J = 14.8, 7.4, 1H), 1.30 (doublet of quintet, J = 14.8, 7.4 Hz, 1H), 0.96 (d, J = 6.7 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.1, 170.7, 160.4, 104.8, 92.1, 42.2, 33.7, 29.8, 26.9, 19.2, 11.4. HRMS (ESI) calcd for $C_{12}H_{18}O_5$ $[M-H]^-$ 241.1081, found 241.1093.

6.2.12. 5-(1-Hydroxy-3,3-dimethylbutylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11l)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (500 mg, 3.47 mmol), 3,3-dimethylbutanoyl chloride (0.49 mL, 3.47 mmol), pyridine (0.71 mL, 6.94 mmol), and CH_2Cl_2 (4.0 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11l** as an off white solid (377 mg, 45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.38 (s, 1H), 3.12 (s, 2H), 1.73 (s, 6H), 1.07 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.9, 170.6, 160.5, 104.4, 93.0, 46.4, 34.0, 30.0, 26.8.

6.2.13. 5-(1-Hydroxy-4-methylpentylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11m)

Prepared using the general procedure above (*Method B*) from Meldrum's acid (500 mg, 3.47 mmol), 4-methylvaleric acid (0.44 mL, 3.47 mmol), diethyl cyanophosphonate (0.58 mL, 3.82 mmol), triethylamine (1.52 mL, 10.7 mmol), and DMF (7.2 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11m** as a yellowish oil (646 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.29 (s, 1H), 3.11–3.03 (m, 2H), 1.74 (s, 6H), 1.72–1.62 (m, 1H), 1.61–1.55 (m, 2H), 0.95 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.8, 170.7, 160.3, 104.9, 91.3, 35.1, 34.1, 28.3, 26.9, 22.4. HRMS (ESI) calcd for $C_{12}H_{18}O_5$ $[M-H]^-$ 241.1154, found 241.073.

6.2.14. 5-(3-Cyclopropyl-1-hydroxypropylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11n)

Adapted from the general procedure above (*Method B*) from Meldrum's acid (350 mg, 2.40 mmol), 3-cyclobutylpropanoic

acid[34] (0.27 g, 2.40 mmol), diethyl cyanophosphonate (0.45 mL, 2.64 mmol), triethylamine (1.04 mL, 7.44 mmol), and DMF (4.0 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11n** as a yellowish oil (38 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.26 (s, 1H), 3.15 (t, J = 7.3 Hz, 2H), 1.70 (s, 6H), 1.58 (q, J = 7.3 Hz, 2H), 0.48–0.39 (m, 3H), 0.08 (q, J = 5.7, 5.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.1, 170.6, 160.2, 104.8, 91.4, 36.0, 31.4, 26.9, 10.8, 4.7. HRMS (ESI) calcd for $C_{12}H_{16}O_5$ $[M-H]^-$ 239.0925, found 239.0917.

6.2.15. 5-(3-Cyclobutyl-1-hydroxypropylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11o)

Prepared using the general procedure above (*Method B*) from Meldrum's acid (1.05 g, 7.26 mmol), 3-cyclobutylpropanoic acid[34] (939 mg, 7.26 mmol), diethyl cyanophosphonate (1.2 mL, 7.99 mmol), triethylamine (3.14 mL, 22.51 mmol), and DMF (13.0 mL). Aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11o** as a yellowish oil (1.31 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.24 (s, 1H), 2.94 (m, 2H), 2.33 (hept, J = 7.9 Hz, 1H), 2.11–1.98 (m, 2H), 1.90–1.70 (m, 4H), 1.71 (s, 6H), 1.63 (pd, J = 9.1, 1.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.4, 170.6, 160.2, 104.8, 91.3, 35.8, 33.9, 33.3, 28.0, 26.9, 18.3. HRMS (ESI) calcd for $C_{13}H_{18}O_5$ $[M-H]^-$ 253.1081, found 253.1077.

6.2.16. 5-(3-Cyclopentyl-1-hydroxypropylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (11p)

Prepared using the general procedure above (*Method A*) from Meldrum's acid (1.50 g, 10.4 mmol), cyclopentane propionyl chloride (1.59 mL, 10.4 mmol), pyridine (2.12 mL, 20.8 mmol), and CH_2Cl_2 (13.0 mL). However, the reaction was stirred at room temperature for 3.5 h instead of 1.5 h for this particular substrate. Aqueous workup and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **11p** as a yellowish oil (2.42 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.27 (s, 1H), 3.09–3.04 (m, 2H), 1.91–1.75 (m, 3H), 1.73 (s, 6H), 1.72–1.67 (m, 2H), 1.66–1.57 (m, 2H), 1.57–1.47 (m, 2H), 1.20–1.08 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.6, 170.7, 160.3, 104.9, 91.3, 40.1, 35.4, 32.6, 27.0, 25.3. HRMS (ESI) calcd for $C_{14}H_{20}O_5$ $[M-H]^-$ 267.1238, found 267.1228.

6.3. General procedure for the synthesis of *N*,*O*-diBoc-protected β-keto hydroxamic acids

Method C: Adapted from the procedure of Sørensen et al.[14] To a solution of acyl Meldrum's acid (0.915 mmol) in toluene (8.5 mL) was added *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine (0.915 mmol). The resulting reaction mixture was stirred at 65 °C for 16 h, and then cooled to room temperature. The solvent was concentrated in vacuo, and the residue was purified on silica gel chromatography using 5% ethyl acetate in hexane to yield the intermediate β-keto hydroxamic acid (**12b**, **c**, **e**, **j**, **k**, **m**–**o**) as colorless oil. In the case of **12e**, and **12m**–**o**, this procedure gave the desired hydroxamic acids as roughly 9:1 mixtures with residual BocNHOBoc. Rather than achieve analytical purity for these compounds, the crude products were used directly in the next step. Thus synthetic details and characterization data for **12e**, and **12m**–**o** are not reported below.

Method D: To a solution of acyl Meldrum's acid (1.69 mmol, 1.8 equiv) in dry toluene (4.5 mL) at room temperature was added *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine (219 mg, 0.938 mmol, 1.0 equiv), and the reaction mixture was immersed in an oil bath preheated to 90 °C. The reaction was monitored for the complete consumption of *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine. Leaving the reaction for longer time can lead to loss in yield and competing side reactions. After concentration in vacuo, the residue was purified on silica gel chromatography using 5% ethyl acetate in hexane to yield β-keto hydroxamic acid (**12a**, **d**, **f**–**i**, **l**, **p**) as colorless oil.

6.3.1. *tert*-Butyl *tert*-butoxycarbonyloxy(3-oxobutanoyl)carbamate (12a)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11a**) (314 mg, 1.69 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (219 mg, 0.94 mmol), and toluene (4.5 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12a** as a clear oil (212 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.11–3.85 (m, 2H), 2.26 (s, 3H), 1.54 (s, 9H), 1.50 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) *of keto tautomer* δ199.9, 163.3, 151.0, 149.7, 86.5, 86.1, 52.6, 30.1, 27.9, 27.6. The *keto*/*enol* ratio for this compound was approximately 6:1.

6.3.2. *tert*-Butyl *tert*-butoxycarbonyloxy(3-cyclopropyl-3-oxopropanoyl)carbamate (12b)

Prepared using the general procedure above (*Method C*) from acyl Meldrum's acid (**11b**) (600 mg, 2.82 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (659 mg, 2.82 mmol), and toluene (26.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12b** as a clear oil (503 mg, 52%). ^{1}H NMR *of keto tautomer* (500 MHz, $CDCl_3$) δ 4.27–3.98 (m, 2H), 2.02–1.96 (m, 1H), 1.53 (s, 9H), 1.50 (s, 9H), 1.15–1.06 (m, 2H), 0.98–0.90 (m, 2H). ^{13}C NMR *of keto tautomer* (126 MHz, $CDCl_3$) δ 202.2, 163.6, 151.0, 149.5, 86.3, 85.9, 52.5, 27.9, 27.6, 20.8, 11.6. The *keto*/*enol* ratio for this compound was approximately 33:1.

6.3.3. *tert*-Butyl *tert*-butoxycarbonyloxy(4-methyl-3-oxopentanoyl)carbamate (12c)

Prepared using the general procedure above (*Method C*) from acyl Meldrum's acid (**11c**) (196 mg, 0.92 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (213 mg, 0.92 mmol), and toluene (8.5 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12c** as a clear oil (100 mg, 32%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.23–3.89 (m, 2H), 2.73 (hept, J = 6.9 Hz, 1H), 1.54 (s, 9H), 1.49 (s, 9H), 1.13 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) *for keto*/*enol tautomeric mixture* δ 206.0, 187.8, 168.7, 163.8, 151.5, 151.0, 149.7, 87.6, 86.3, 86.2, 85.8, 85.3, 50.1, 41.1, 34.9, 28.0, 27.9, 27.7, 27.6, 19.9, 18.1. The *keto*/*enol* ratio for this compound was approximately 4:1.

6.3.4. *tert*-Butyl *tert*-butoxycarbonyloxy(3-cyclobutyl-3-oxopropanoyl)carbamate (12d)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11d**) (522 mg, 2.30 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (298 mg, 1.28 mmol), and toluene (6.4 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12d** as a clear oil (crude) contaminated with residual *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine as the impurity (389 mg, 85% (crude)). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.13–3.77 (m, 2H), 3.37 (p, J = 9.0 Hz, 1H), 2.37–2.22 (m, 2H), 2.21–2.10 (m, 2H), 2.01–1.90 (m, 1H), 1.88–1.77 (m, 1H), 1.54 (s, 9H), 1.49 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 203.2, 184.7, 168.6, 163.6, 151.5, 151.0, 149.6, 149.4, 88.2, 86.4, 86.2, 85.9, 85.3, 53.6, 49.6, 45.6, 40.1, 27.9, 27.7, 26.1, 24.5, 18.3, 17.9. HRMS (ESI) calcd for $C_{17}H_{27}NO_7$ $[M+Na]^+$ 380.1680, found 380.1706. The *keto*/*enol* ratio for this compound was approximately 5:1.

6.3.5. *tert*-Butyl *tert*-butoxycarbonylox(3-cyclopentyl-3-oxopropanoyl)carbamate (12f)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11f**) (93.3 mg, 0.39 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (50.0 mg, 0.22 mmol), and toluene (1.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12f** as a clear oil (59.0 mg, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.22–3.89 (m, 2H), 2.98 (p, J = 8.0 Hz, 1H), 1.91–1.76 (m, 4H), 1.70–1.63 (m, 2H), 1.62–1.56 (m, 2H), 1.54 (s, 9H), 1.49 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) *of keto/enol tautomeric mixture* δ 204.7, 186.6, 168.5, 163.7, 151.5, 151.0, 149.6, 149.4, 88.4, 86.3, 86.2, 85.8, 85.3, 51.7, 51.2, 45.9, 30.9, 28.9, 28.0, 27.9, 27.7, 27.6, 26.0, 26.0. HRMS (ESI) calcd for $C_{18}H_{29}NO_7$ $[M+Na]^+$ 394.1836, found 394.1868. The *keto*/*enol* ratio for this compound was approximately 6:1.

6.3.6. *tert*-Butyl *tert*-butoxycarbonyloxy(4-methyl-3-oxoheptanoyl)carbamate (12g)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11g**) (278 mg, 1.15 mmol), *N*,*O*-bis-(*tert*-butoxycarbonyl)-hydroxylamine (149 mg, 0.64 mmol), and toluene (3.1 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12g** as a clear oil (163 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.22–3.88 (m, 2H), 2.65 (h, J = 6.7 Hz, 1H), 1.73–1.62 (m, 2H), 1.54 (s, 9H), 1.49 (s, 9H), 1.40–1.26 (m, 2H), 1.11 (d, J = 7.0 Hz, 3H), 0.89 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) *of keto/enol tautomer* δ205.9, 187.3, 168.6, 163.7, 151.5, 151.0, 149.7, 149.4, 88.7, 86.3, 86.2, 85.8, 85.3, 50.6, 46.2, 40.4, 36.6, 34.8, 28.0, 27.9, 27.7, 27.6, 20.6, 20.3, 18.2, 15.9, 14.2, 14.1. HRMS (ESI) calcd for $C_{18}H_{31}NO_7$ $[M+H_2O]$ 391.21, found 391.25. The *keto*/*enol* ratio for this compound was approximately 6:1.

6.3.7. *tert*-Butyl *tert*-butoxycarbonyloxy(4-ethyl-3-oxohexanoyl)carbamate (12h)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11h**) (942 mg, 3.9 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (504 mg, 2.16 mmol), and toluene (11.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12h** as a clear oil (536 mg, 66%). ^{1}H NMR *of keto*/*enol tautomeric mixture* (500 MHz, $CDCl_3$) δ 13.13 (s, 1H), 6.14 (s, 1H), 4.29–3.80 (m, 2H), 2.49–2.42 (m, 1H), 2.00–1.92 (m, 1H), 1.75–1.55 (m, 4H), 1.55 (s, 9H), 1.54 (s, 9H), 1.53 (s, 9H), 1.49 (s, 9H), 0.91–0.84 (m, 6H). ^{13}C NMR *of keto/enol tautomer* (126 MHz, $CDCl_3$) δ 205.4, 185.9, 168.4, 163.7, 151.5, 151.0, 149.6, 149.3, 90.4, 86.3, 86.2, 85.7, 85.3, 55.0, 51.3, 50.2, 28.0, 27.9, 27.7, 27.6, 25.7, 23.3, 12.1, 11.5. HRMS (ESI) calcd for $C_{18}H_{31}NO_7$ $[M+Na]^+$ 396.1993, found 396.197. The *keto*/*enol* ratio for this compound was approximately 2:1.

6.3.8. *tert*-Butyl *tert*-butoxycarbonyloxy(4-ethyl-3-oxooctanoyl)carbamate (12i)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11i**) (54.6 mg, 0.20 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (26.1 mg, 0.11 mmol), and toluene (0.9 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12i** as a clear oil (30.2 mg, 66%). ^{1}H NMR *of keto*/*enol tautomeric mixture* (500 MHz, $CDCl_3$) δ 13.14 (s, 0.5H), 6.13 (s, 0.5H), 4.41–3.63 (m, 2H), 2.50 (p, J = 6.5, 5.9 Hz, 1H), 2.07–2.00 (m, 0.5H), 1.80–1.57 (m, 4H), 1.55 (s, 4.5H), 1.54 (s, 9H), 1.53 (s, 4.5H), 1.49 (s, 9H), 1.48–1.35 (m, 2H), 1.34–1.15 (m, 7H), 0.91–0.84 (m, 9H). ^{13}C NMR *of keto*/*enol tautomer* (126 MHz, $CDCl_3$) δ 205.5, 186.1, 168.5, 163.7, 151.5, 151.0, 149.6, 149.3, 90.3, 86.3, 86.2, 85.7, 85.3, 53.6, 51.3, 48.6, 32.5, 30.2, 29.7, 29.3, 28.1, 27.9, 27.7, 27.7, 26.1, 23.9, 23.0, 22.9, 14.1, 14.1, 12.1, 11.6. HRMS (ESI) calcd for $C_{20}H_{35}NO_7$ $[M+Na]^+$ 424.2306, found 424.2343. The *keto*/*enol* ratio for this compound was approximately 2:1.

6.3.9. *tert*-Butyl *tert*-butoxycarbonyloxy(5-methyl-3-oxohexanoyl)carbamate (12j)

Prepared using the general procedure above (*Method C*) from acyl Meldrum's acid (**11j**) (239 mg, 1.05 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (244 mg, 1.05 mmol), and toluene (9.7 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12j** as a clear oil (125 mg, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.16–3.74 (m, 2H), 2.41 (d, J = 6.8 Hz, 2H), 2.22–2.11 (m, 1H), 1.54 (s, 9H), 1.49 (s, 9H), 0.93 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) *for keto*/*enol tautomeric mixture* δ 201.6, 182.3, 168.3, 163.3, 151.3, 150.8, 149.5, 149.2, 90.5, 86.2, 86.0, 85.7, 85.2, 52.2, 51.5, 45.2, 27.9, 27.8, 27.5, 27.5, 26.6, 24.1, 22.4. HRMS (ESI) calcd for $C_{17}H_{29}NO_7$ $[M+Na]^+$ 382.1836, found 382.869. The *keto*/*enol* ratio for this compound was approximately 4:1.

6.3.10. *tert*-Butyl *tert*-butoxycarbonyloxy(5-methyl-3-oxoheptanoyl)carbamate (12k)

Prepared using the general procedure above (*Method C*) from acyl Meldrum's acid (**11k**) (380 mg, 1.57 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (366 mg, 1.57 mmol), and toluene (14.5 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12k** as a clear oil (191 mg, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.25–3.66 (m, 2H), 2.59–2.44 (m, 1H), 2.39–2.18 (m, 1H), 1.95 (m, J = 13.4, 6.7 Hz, 1H), 1.54 (s, 9H), 1.49 (s, 9H), 1.42–1.27 (m, 1H), 1.26–1.12 (m, 1H), 0.90 (d, J = 6.7 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) *for keto*/*enol tautomeric mixture* δ 201.8, 182.6, 168.2, 163.4, 151.3, 150.8, 149.5, 149.2, 90.6, 86.2, 86.1, 85.7, 85.2, 52.2, 49.7, 43.3, 32.8, 30.2, 29.4, 29.3, 27.9, 27.8, 27.5, 27.5, 19.3, 19.0, 11.3, 11.2. HRMS (ESI) calcd for $C_{18}H_{31}NO_7$ $[M+Na]^+$ 396.1993, found 396.2007. The *keto*/*enol* ratio for this compound was approximately 4:1.

6.3.11. *tert*-Butyl *tert*-butoxycarbonyloxy(5,5-dimethyl-3-oxohexanoyl)carbamate (12l)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11l**) (362 mg, 1.49 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (194 mg, 0.83 mmol), and toluene (4.2 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12l** as a clear oil (242 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.14–3.67 (m, 2H), 2.44 (s, 2H), 1.54 (s, 9H), 1.49 (s, 9H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) *of keto*/*enol tautomeric mixture* δ 201.4, 182.0, 168.4, 163.5, 151.5, 151.0, 149.7, 149.3, 91.8, 86.3, 86.2, 85.8, 85.3, 54.9, 53.6, 49.9, 31.9, 30.9, 30.0, 29.6, 28.0, 27.9, 27.7, 27.7. The *keto*/*enol* ratio for this compound was approximately 2:1.

6.3.12. *tert*-Butyl *tert*-butoxycarbonyloxy(5-cyclopentyl-3-oxopentanoyl)carbamate (12p)

Prepared using the general procedure above (*Method D*) from acyl Meldrum's acid (**11p**) (2.32 g, 8.65 mmol), *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (2.02 g, 8.65 mmol), and toluene (80.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (5% ethyl acetate in hexane) yielded **12p** as a clear oil (1.50 g, 43%). ^{1}H NMR (500 MHz, $CDCl_3$) *of keto tautomer* δ 4.26–3.54 (m, 2H), 2.57–2.50 (m, 2H),

1.81–1.70 (m, 3H), 1.65–1.57 (m, 6H), 1.54 (s, 9H), 1.49 (s, 9H), 1.15–1.00 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ *of keto tautomer* 202.2, 163.4, 150.8, 149.5, 86.2, 85.8, 51.8, 41.9, 39.4, 32.4, 29.4, 27.8, 27.5, 25.1. HRMS (ESI) calcd for $C_{20}H_{33}NO_7$ $[M+Na]^+$ 422.2149, found 422.2172. The *keto*/*enol* ratio for this compound was approximately 6:1.

6.4. General procedure for the synthesis of 5-substituted isoxazol-3-ols 13a–p

Method E: Adapted from the procedure of Sørensen et al.[14] β-keto hydroxamic acid (1.33 mmol) was dissolved in methanol (3.0 mL), and added drop wise to concentrated HCl (5.0 mL) pre-heated to 50 °C. The reaction mixture was stirred for 2.5 h at 50 °C prior to being brought to room temperature. The reaction mixture was concentrated in vacuo. The residual reaction mixture was partitioned between water and dichloromethane. The aqueous layer was extracted twice with dichloromethane and the combined organic layers were dried with sodium sulfate. The solvent was evaporated, and the subsequent residue was purified on silica gel chromatography using a gradient from 5% to 10% ethyl acetate in hexane, 1% acetic acid to yield isoxazol-3-ols.

6.4.1. 5-Methylisoxazol-3-ol (13a)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12a**) (66.5 mg, 0.21 mmol), concentrated HCl (1.0 mL), and methanol (0.5 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13a** as a white solid (15.5 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) 11.57 (s, 1H), 5.67 (s, 1H), 2.33 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 170.4, 94.0, 13.0.

6.4.2. 5-Cyclopropylisoxazol-3-ol (13b)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12b**) (2.69 g, 7.83 mmol), concentrated HCl (25.0 mL), and methanol (16.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13b** as an off white solid (464 mg, 47%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.94 (s, 1H), 5.57 (s, 1H), 1.95–1.86 (m, 1H), 1.08–1.00 (m, 2H), 0.97–0.91 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.6, 171.5, 91.1, 8.6, 8.3.

6.4.3. 5-Isopropylisoxazol-3-ol (13c)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12b**) (973 mg, 2.82 mmol), concentrated HCl (12.0 mL), and methanol (6.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13c** as a white solid (276 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.17 (s, 1H), 5.64 (s, 1H), 2.94 (hept, J = 6.9 Hz, 1H), 1.27 (d, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.7, 171.2, 91.5, 27.7, 20.6.

6.4.4. 5-Cyclobutylisoxazol-3-ol (13d)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12d**) (318 mg, 0.89 mmol), concentrated HCl (3.1 mL), and methanol (2.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13d** as a semi-solid (75.0 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.68 (s, 1H), 3.50 (p, J = 8.5 Hz, 1H), 2.40–2.31 (m, 2H), 2.30–2.20 (m, 2H), 2.10–2.01 (m, 1H), 2.01–1.91 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ177.4, 171.2, 91.9, 32.6, 27.8, 18.9. HRMS (ESI) calcd for $C_7H_9NO_2$ $[M+H]^+$ 140.0706, found 140.0693.

6.4.5. 5-*sec*-Butylisoxazol-3-ol (13e)

Prepared using the general procedure above (*Method E*) from crude β-keto hydroxamic acid (**12e**) (1.96 g, 5.53 mmol), concentrated HCl (20.0 mL), and methanol (15.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13e** as an off white solid (620 mg, 77%); mp 37.0–38.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.41 (s, 1H), 5.64 (s, 1H), 2.74 (h, J = 7.0 Hz, 1H), 1.74–1.64 (m, 1H), 1.58 (doublet of quintet, J = 14.1, 7.2 Hz, 1H), 1.24 (d, J = 7.0 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.8, 171.2, 92.1, 34.5, 28.2, 18.1, 11.4. HRMS (ESI) calcd for $C_7H_{11}NO_2$ $[M+H]^+$ 142.0863, found 142.086.

6.4.6. 5-Cyclopentylisoxazol-3-ol (13f)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12f**) (1.77 g, 4.76 mmol), concentrated HCl (25.0 mL), and methanol (10.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13f** as a white solid (508 mg, 70%); mp 71.3–73.4 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.42 (s, 1H), 5.63 (s, 1H), 3.06 (quintet, J = 7.9 Hz, 1H), 2.09–1.96 (m, 2H), 1.80–1.61 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.4, 171.2, 91.8, 38.0, 31.6, 25.4. HRMS (ESI) calcd for $C_8H_{11}NO_2$ $[M+H]^+$ 154.0863, found 154.0855.

6.4.7. 5-(Pentan-2-yl)isoxazol-3-ol (13g)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12g**) (2.85 g, 7.63 mmol), concentrated HCl (15.0 mL), and methanol (16.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13g** as a colorless oil (889 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.20 (s, 1H), 5.62 (s, 1H), 2.82 (h, J = 7.0 Hz, 1H), 1.64 (ddt, J = 13.5, 9.4, 6.6 Hz, 1H), 1.50 (ddt, J = 13.5, 9.4, 6.6 Hz, 1H), 1.38–1.26 (m, 2H), 1.23 (d, J = 7.1 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.0, 171.2, 91.8, 37.4, 32.7, 20.2, 18.6, 13.9. HRMS (ESI) calcd for $C_8H_{13}NO_2$ $[M+H]^+$ 156.0946, found 156.1485.

6.4.8. 5-(Pentan-3-yl)isoxazol-3-ol (13h)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12h**) (496 mg, 7.63 mmol), concentrated HCl (5.0 mL), and methanol (3.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13h** an off white solid (115 mg, 81%); mp 49.0–50.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H), 5.65 (s, 1H), 2.55 (tt, J = 8.3, 5.8 Hz, 1H), 1.72–1.56 (m, 4H), 0.87 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.7, 171.1, 93.0, 42.2, 26.3, 11.7. HRMS (ESI) calcd for $C_8H_{13}NO_2$ $[M+H]^+$ 156.1019, found 156.1013.

6.4.9. 5-(Heptan-3-yl)isoxazol-3-ol (13i)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12i**) (2.29 g, 5.70 mmol), concentrated HCl (22.0 mL), and methanol (12.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13i** as a colorless oil (396 mg, 40%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (s, 1H), 2.62 (quintet, J = 7.4 Hz, 1H), 1.74–1.53 (m, 4H), 1.39–1.12 (m, 6H), 0.95–0.81 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.1, 171.2, 93.0, 40.6, 33.2, 29.4, 26.8, 22.7, 14.1, 11.7. HRMS (ESI) calcd for $C_{10}H_{17}NO_2$ $[M+H]^+$ 184.1332, found 184.1339.

6.4.10. 5-Isobutylisoxazol-3-ol (13j)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12j**) (2.98 g, 8.67 mmol), concentrated HCl (25.0 mL), and methanol (18.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13j** as an off white solid (804 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.80 (s, 1H), 5.66 (s, 1H), 2.50 (d, J = 7.1 Hz, 2H), 2.06–1.94 (m, 1H), 0.95 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.8, 171.3, 93.8, 36.3, 27.6, 22.3.

6.4.11. 5-(2-Methylbutyl)isoxazol-3-ol (13k)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12k**) (3.09 g, 8.27 mmol), concentrated HCl (30.0 mL), and methanol (17.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13k** as an oil (871 mg, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.87 (s, 1H), 5.66 (s, 1H), 2.62 (dd, J = 14.9, 6.1 Hz, 1H), 2.45 (dd, J = 14.9, 7.9 Hz, 1H), 1.84–1.71 (m, J = 6.6 Hz, 1H), 1.46–1.33 (m, 1H), 1.23 (dq, J = 21.1, 7.5 Hz, 2H), 0.92 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.9, 171.3, 94.0, 34.4, 33.9, 29.2, 19.2, 11.4. HRMS (ESI) calcd for $C_8H_{13}NO_2$ $[M+H]^+$ 156.1019, found 156.1013.

6.4.12. 5-Neopentylisoxazol-3-ol (13l)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12l**) (2.29 g, 6.40 mmol), concentrated HCl (20.0 mL), and methanol (14.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13l** as an off white solid (664 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.67 (s, 1H), 2.52 (s, 2H), 0.98 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 171.1, 94.9, 41.6, 31.7, 29.6. HRMS (ESI) calcd for $C_8H_{13}NO_2$ $[M+H]^+$ 156.1019, found 156.1021.

6.4.13. 5-Isopentylisoxazol-3-ol (13m)

Prepared using the general procedure above (*Method E*) from crude β-keto hydroxamic acid (**12m**) (1.64 g, 4.39 mmol), concentrated HCl (11.0 mL), and methanol (9.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13m** as a white solid (629 mg, 92%); mp 53.0–54.8. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (s, 1H), 5.65 (s, 1H), 2.65–2.61 (m, 2H), 1.66–1.49 (m, 3H), 0.93 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.0, 171.3, 93.0, 36.1, 27.7, 25.4, 22.3. HRMS (ESI) calcd for $C_8H_{13}NO_2$ $[M+H]^+$ 156.1019, found 156.1011.

6.4.14. 5-(2-Cyclopropylethyl)isoxazol-3-ol (13n)

Prepared using the general procedure above (*Method E*) from crude β-keto hydroxamic acid (1.45 g), concentrated HCl (21.0 mL), and methanol (9.5 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13n** as a slight yellow crystalline solid (290 mg, 34% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.73 (s, 1H), 5.67 (s, 1H), 2.73 (t, J = 7.6 Hz, 2H), 1.56 (q, J = 7.3 Hz, 2H), 0.79–0.63 (m, 1H), 0.54–0.35 (m, 2H), 0.06 (dt, J = 5.9, 4.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 171.3, 93.2, 32.5, 27.6, 10.6, 4.6. HRMS (ESI) calcd for $C_8H_{11}NO_2$ $[M+H]^+$ 154.0863, found 154.0856.

6.4.15. 5-(2-Cyclobutylethyl)isoxazol-3-ol (13o)

Prepared using the general procedure above (*Method E*) from crude β-keto hydroxamic acid (1.18 g), concentrated HCl (7.5 mL), and methanol (16.5 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13o** as a white crystalline solid (340 mg, 42% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.81 (s, 1H), 5.63 (s, 1H), 2.52 (t, J = 7.7 Hz, 2H), 2.28 (hept, J = 8.1 Hz, 1H), 2.10–1.98 (m, 2H), 1.92–1.76 (m, 2H), 1.73 (q, J = 7.6 Hz, 2H), 1.60 (pd, J = 9.0, 2.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.6, 171.3, 93.0, 35.4, 34.3, 28.0, 25.1, 18.4. HRMS (ESI) calcd for $C_9H_{13}NO_2$ $[M+H]^+$ 168.1019, found 168.1022.

6.4.16. 5-(2-Cyclopentylethyl)isoxazol-3-ol (13p)

Prepared using the general procedure above (*Method E*) from β-keto hydroxamic acid (**12p**) (1.50 g, 3.75 mmol), concentrated HCl (13.0 mL), and methanol (8.0 mL). Concentration of the reaction mixture in vacuo followed by an aqueous work up, and silica gel chromatography (5–10% ethyl acetate in hexane, 1% acetic acid) afforded **13p** as a white solid (521 mg, 77%); mp 67.0–68.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (s, 1H), 2.67–2.59 (m, 2H), 1.84–1.75 (m, 3H), 1.70–1.64 (m, 2H), 1.64–1.49 (m, 4H), 1.17–1.01 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.9, 171.3, 93.0, 39.6, 33.6, 32.6, 26.7, 25.3. HRMS (ESI) calcd for $C_{10}H_{15}NO_2$ $[M+H]^+$ 182.1176, found 182.1182.

6.5. General procedure for the synthesis of *N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide 14a–h, j–p

Method F: To solution of isoxazol-3-ol (0.40 mmol) in toluene (2.0 mL) was added *N*,*N*-dimethylcarbamoyl chloride (0.19 mL, 2.00 mmol) under nitrogen atmosphere. The resulting reaction mixture was refluxed overnight. The reaction mixture was cooled to room temperature and solvent was evaporated in vacuo. The resulting residue was purified by flash column chromatography on silica gel using a gradient of 20–30% ethyl acetate in hexane to yield carboxamide as the major product and carbamate as the minor product.

6.5.1. *N*,*N*,5-Trimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14a)

Prepared using the general procedure above (*Method F*) from 5-methylisoxazol-3-ol (**13a**) (45.7 mg, 0.46 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.21 mL, 2.31 mmol), and toluene (2.5 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14a** as an off white solid (66.3 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.41 (s, 1H), 3.10 (br s, 6H), 2.31 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.5, 167.9, 150.2, 96.9, 38.8, 37.0, 13.9. HRMS (ESI) calcd for $C_7H_{10}N_2O_3$ $[M+H]^+$ 171.0691, found 171.0525.

6.5.2. 5-Cyclopropyl-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14b)

Prepared using the general procedure above (*Method F*) from 5-cyclopropylisoxazol-3-ol (**13b**) (20.5 mg, 0.16 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.08 mL, 0.82 mmol), and toluene (1.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14b** as a clear oil (4.60 mg, 14%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.27 (s, 1H), 3.07 (br s, 6H), 1.91–1.81 (m, 1H), 1.19–1.00 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.0, 168.3, 150.4, 92.9, 38.8, 36.9, 9.3, 9.3. HRMS (ESI) calcd for $C_9H_{12}N_2O_3$ $[M+H]^+$ 197.0921, found 197.0933.

6.5.3. 5-Isopropyl-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14c)

Prepared using the general procedure above (*Method F*) from 5-isopropylisoxazol-3-ol (**13c**) (136 mg, 1.07 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.49 mL, 5.34 mmol), and toluene (6.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded

14c as a white solid (129 mg, 61%); mp 94.0–95.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (s, 1H), 3.10 (br s, 6H), 2.88 (hept, J = 6.7 Hz, 1H), 1.29 (d, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 183.2, 168.0, 150.3, 94.1, 38.8, 37.1, 28.2, 19.9. HRMS (ESI) calcd for $C_9H_{14}N_2O_3$ $[M+H]^+$ 198.1004, found 199.1083.

6.5.4. 5-Cyclobutyl-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14d)

Prepared using the general procedure above (*Method F*) from 5-cyclobutylisoxazol-3-ol (**13d**) (63.0 mg, 0.45 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.21 mL, 2.26 mmol), and toluene (2.2 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14d** as a white solid (75.0 mg, 79%); mp 62.5–64.3 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 (s, 1H), 3.44 (quintet, J = 8.6 Hz, 1H), 3.10 (br s, 6H), 2.42–2.33 (m, 2H), 2.32–2.22 (m, 2H), 2.13–2.02 (m, 1H), 2.02–1.90 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.9, 167.9, 150.3, 94.5, 38.8, 37.0, 32.8, 27.1, 18.9. HRMS (ESI) calcd for $C_{10}H_{14}N_2O_3$ $[M+H]^+$ 211.1077, found 211.1068.

6.5.5. 5-*sec*-Butyl-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14e)

Prepared using the general procedure above (*Method F*) from 5-*sec*-butylisoxazol-3-ol (**13e**) (96.0 mg, 0.68 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.31 mL, 3.38 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14e** as clear oil (104 mg, 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (s, 1H), 3.09 (br s, 6H), 2.67 (hextet, J = 6.9 Hz, 1H), 1.71 (doublet of quintets, J = 14.3, 7.3 Hz, 1H), 1.59 (doublet of quintets, J = 14.3, 7.3 Hz, 1H), 1.25 (d, J = 7.0 Hz, 8H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.7, 168.1, 150.3, 94.7, 38.8, 35.0, 27.5, 17.3, 11.3. HRMS (ESI) calcd for $C_{10}H_{16}N_2O_3$ $[M+H]^+$ 213.1234, found 213.1238.

6.5.6. 5-Cyclopentyl-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14f)

Prepared using the general procedure above (*Method F*) from 5-cyclopentylisoxazol-3-ol (**13f**) (21.0 mg, 0.14 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.06 mL, 0.69 mmol), and toluene (1.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14f** as a white solid (21.0 mg, 67%); mp 69.0–71.8 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (s, 1H), 3.10 (br s, 6H), 3.04–2.96 (m, 1H), 2.14–1.99 (m, 2H), 1.86–1.60 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.3, 168.0, 150.3, 94.4, 38.4, 31.2, 25.5. HRMS (ESI) calcd for $C_{11}H_{16}N_2O_3$ $[M+Na]^+$ 225.1234, found 225.1212. Due to signal broadening (slow chemical exchange), the carboxamide methyls could not be detected by ^{13}C NMR, though they were clearly visible in the ^{1}H NMR spectrum (see Fig. 2 for explanation).

6.5.7. *N*,*N*-Dimethyl-3-oxo-5-(pentan-2-yl)isoxazole-2(3*H*)-carboxamide (14g)

Prepared using the general procedure above (*Method F*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (98.0 mg, 0.63 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.17 mL, 1.59 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14g** as a clear oil (110 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (s, 1H), 3.10 (br s, 6H), 2.75 (hextet, J = 6.9 Hz, 1H), 1.71–1.64 (m, 1H), 1.56–1.47 (m, 1H), 1.44–1.31 (m, 2H), 1.26 (d, J = 7.0 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.9, 168.1, 150.3, 94.6, 36.7, 33.3, 20.2, 17.8, 14.0. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+H]^+$ 227.139, found 227.1385. Due to signal broadening (slow chemical exchange), the carboxamide methyls could not be detected by ^{13}C NMR, though they were clearly visible in the ^{1}H NMR spectrum (see Fig. 2 for explanation).

6.5.8. *N*,*N*-Dimethyl-3-oxo-5-(pentan-3-yl)isoxazole-2(3*H*)-carboxamide (14h)

Prepared using the general procedure above (*Method F*) from 5-(pentan-3-yl)isoxazol-3-ol (**13h**) (62.1 mg, 0.40 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.18 mL, 2.00 mmol), and toluene (2.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14h** as a clear oil (73.4 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (s, 1H), 3.10 (br s, 6H), 2.52–2.44 (m, 1H), 1.74–1.57 (m, 4H), 0.94 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.8, 168.2, 150.3, 95.7, 42.6, 37.0, 25.6, 11.6. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+Na]^+$ 249.121, found 249.1213.

6.5.9. 5-Isobutyl-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14j)

Prepared using the general procedure above (*Method F*) from 5-isobutylisoxazol-3-ol (**13j**) (49.0 mg, 0.35 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.16 mL, 1.74 mmol), and toluene (1.7 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14j** as a clear oil (40.0 mg, 54%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (s, 1H), 3.10 (br s, 6H), 2.47 (d, J = 7.1 Hz, 2H), 2.12–1.97 (m, 1H), 1.00 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.7, 168.0, 150.3, 96.7, 36.9, 27.1, 22.4. Due to signal broadening (slow chemical exchange), the carboxamide methyls could not be detected by ^{13}C NMR, though they were clearly visible in the ^{1}H NMR spectrum (see Fig. 2 for explanation). HRMS (ESI) calcd for $C_{10}H_{16}N_2O_3$ $[M+H]^+$ 213.1509, found 213.1261.

6.5.10. *N*,*N*-Dimethyl-5-(2-methylbutyl)-3-oxoisoxazole-2(3*H*)-carboxamide (14k)

Prepared using the general procedure above (*Method F*) from 5-(2-methylbutyl)isoxazol-3-ol (**13k**) (29.0 mg, 0.19 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.09 mL, 0.94 mmol), and toluene (1.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14k** as a clear oil (19.3 mg, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (s, 1H), 3.10 (br s, 6H), 2.58 (dd, J = 15.0, 7.0 Hz, 1H), 2.39 (dd, J = 15.0, 7.0 Hz, 1H), 1.80 (doublet of quintets, J = 13.5, 6.7 Hz, 1H), 1.48–1.35 (m, 1H), 1.32–1.18 (m, 1H), 0.97 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.9, 168.0, 150.3, 96.7, 39.0, 37.1, 35.0, 33.3, 29.3, 19.2, 11.3. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+H]^+$ 227.139, found 227.1403.

6.5.11. *N*,*N*-Dimethyl-5-neopentyl-3-oxoisoxazole-2(3*H*)-carboxamide (14l)

Prepared using the general procedure above (*Method F*) from 5-neopentylisoxazol-3-ol (**13l**) (40.0 mg, 0.28 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.13 mL, 1.41 mmol), and toluene (1.4 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14l** as a white solid (52.0 mg, 81%); mp 70.7–72.0. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (s, 1H), 3.11 (br s, 6H), 2.48 (s, 2H), 1.03 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.0, 168.2, 150.3, 97.7, 42.0, 38.8, 36.8, 31.8, 29.6. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+H]^+$ 227.139, found 227.1383.

6.5.12. 5-Isopentyl-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14m)

Prepared using the general procedure above (*Method F*) from 5-isopentylisoxazol-3-ol (**13m**) (100 mg, 0.64 mmol), *N*,*N*-dimethyl-

carbamoyl chloride (0.30 mL, 3.22 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14m** as a clear oil (122 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (s, 1H), 3.08 (br s, 6H), 2.57 (t, J = 7.9 Hz, 2H), 1.70–1.50 (m, 3H), 0.92 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.9, 168.0, 150.3, 95.7, 38.8, 37.0, 35.1, 27.7, 26.0, 22.2. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+H]^+$ 227.1390, found 227.1397.

6.5.13. 5-(2-Cyclopropylethyl)-*N*,*N*-dimethyl-3-oxoisoxazole-2(3H)-carboxamide (14n)

Prepared using the general procedure above (*Method F*) from 5-(2-cyclopropylethyl)isoxazol-3-ol (**13n**) (74 mg, 0.48 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.23 mL, 2.41 mmol), and toluene (2.5 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14n** as a clear liquid (78 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.39 (s, 1H), 3.08 (br s, 6H), 2.67 (t, J = 7.9 Hz, 2H), 1.56 (q, J = 7.3 Hz, 2H), 0.80–0.65 (m, 1H), 0.53–0.40 (dq, J = 8.3, 2.67 Hz, 3H), 0.07 (q, J = 4.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.4, 167.9, 150.3, 96.0, 38.7, 37.1, 31.5, 28.2, 10.5, 4.7. HRMS (ESI) calcd for $C_{11}H_{16}N_2O_3$ $[M+H]^+$ 225.1234, found 225.1231.

6.5.14. 5-(2-Cyclobutylethyl)-*N*,*N*-dimethyl-3-]oxoisoxazole-2(3*H*)-carboxamide (14o)

Prepared using the general procedure above (*Method F*) from 5-(2-cyclobutylethyl)isoxazol-3-ol (**13o**) (90 mg, 0.54 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.25 mL, 2.72 mmol), and toluene (3.5 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14o** as a clear liquid (87 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (s, 1H), 3.05 (br s, 6H), 2.44 (td, J = 7.8, 0.7 Hz, 3H), 2.27 (hept, J = 7.8 Hz, 1H), 2.11–1.95 (m, 2H), 1.89–1.74 (m, 2H), 1.71 (q, J = 7.7 Hz, 2H), 1.64–1.51 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.5, 167.8, 150.2, 95.7, 38.7, 36.9, 35.2, 33.2, 27.9, 27.9, 25.7, 18.2. HRMS (ESI) calcd for $C_{12}H_{18}N_2O_3$ $[M+H]^+$ 239.1390, found 239.1387.

6.5.15. 5-(2-Cyclopentylethyl)-*N*,*N*-dimethyl-3-oxoisoxazole-2(3*H*)-carboxamide (14p)

Prepared using the general procedure above (*Method F*) from 5-(2-cyclopentylethyl)isoxazol-3-ol (**13p**) (77.8 mg, 0.43 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.20 mL, 2.15 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **14p** as a white solid (85.0 mg, 79%); mp 47.8–47.9 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (s, 1H), 3.11 (br s, 6H), 2.59 (t, J = 7.9 Hz, 2H), 1.87–1.74 (m, 3H), 1.71–1.65 (m, 2H), 1.65–1.61 (m, 2H), 1.58–1.49 (m, 2H), 1.18–1.01 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.8, 167.9, 150.3, 95.8, 39.6, 38.8, 36.9, 32.6, 32.5, 27.3, 25.2. HRMS (ESI) calcd for $C_{13}H_{20}N_2O_3$ $[M+H]^+$ 253.1547, found 253.1547.

6.6. General procedure for the synthesis of synthesis of isoxazol-3-yl dimethylcarbamate 15a–p

Method G: To a dry round bottom flask was added isoxazol-3-ol (0.47 mmol) and dry tetrahydrofuran (3.0 mL) prior to cooling it to 0 °C. To this solution was added potassium *tert*-butoxide (1 M in THF, 0.71 mL, 0.71 mmol) at 0 °C and subsequently the reaction mixture was stirred for 20 min at rt. To this solution was added *N*,*N*-dimethyl carbamoyl chloride (0.11 mL, 1.18 mmol), and the resulting solution was stirred at room temperature for additional 16 h. The solvent was concentrated and the residue was taken up in dichloromethane and washed with 0.25 M HCl hydrochloric acid, and then brine, and dried over sodium sulfate. The solvent was evaporated and the residue was purified by silica gel column chromatography using 20% ethyl acetate in hexane to afford the dimethyl carbamate as the major product.

6.6.1. 5-Methylisoxazol-3-yl dimethylcarbamate (15a)

Prepared using the general procedure above (*Method G*) from 5-methylisoxazol-3-ol (**13a**) (200 mg, 2.01 mmol), potassium *tert*-butoxide (2.62 mL, 2.62 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.46 mL, 5.04 mmol), and THF (7.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15a** as a clear oil (256 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.09 (s, 1H), 3.04 (s, 3H), 2.95 (s, 3H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.9, 166.7, 151.8, 96.2, 36.8, 36.6, 13.1. HRMS (ESI) calcd for $C_7H_{10}N_2O_3$ $[M+H]^+$ 171.0691, found 171.0771.

6.6.2. 5-Cyclopropylisoxazol-3-yl dimethylcarbamate (15b)

Prepared using the general procedure above (*Method F*) from 5-cyclopropylisoxazol-3-ol (**13b**) (20.5 mg, 0.16 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.08 mL, 0.82 mmol), and toluene (1.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **15b** as a clear oil (25.0 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.07 (s, 1H), 3.08 (s, 3H), 3.00 (s, 3H), 1.98 (tt, J = 8.5, 5.1 Hz, 1H), 1.07–1.01 (m, 2H), 1.01–0.95 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ176.2, 166.8, 151.9, 93.3, 37.0, 36.7, 8.8, 8.3. HRMS (ESI) calcd for $C_9H_{12}N_2O_3$ $[M+H]^+$ 197.0921, found 197.0921.

6.6.3. 5-Isopropylisoxazol-3-yl dimethylcarbamate (15c)

Prepared using the general procedure above (*Method G*) from 5-isopropylisoxazol-3-ol (**13c**) (122 mg, 0.96 mmol), potassium *tert*-butoxide (1.24 mL, 1.25 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.22 mL, 2.39 mmol), and THF (7.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15c** as a clear oil (170 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.12 (s, 1H), 3.09 (s, 3H), 3.06–2.96 (m, 1H), 3.00 (s, 3H), 1.29 (d, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.2, 166.7, 152.0, 93.7, 37.0, 36.7, 27.9, 20.6. HRMS (ESI) calcd for $C_9H_{14}N_2O_3$ $[M+H]^+$ 198.1004, found 199.1079.

6.6.4. 5-Cyclobutylisoxazol-3-yl dimethylcarbamate (15d)

Prepared using the general procedure above (*Method G*) from 5-cyclobutylisoxazol-3-ol (**13d**) (39.0 mg, 0.28 mmol), potassium *tert*-butoxide (0.42 mL, 0.42 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.06 mL, 0.70 mmol), and THF (2.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15d** as a clear oil (52.0 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 (s, 1H), 3.57 (quintet, J = 8.5 Hz, 1H), 3.09 (s, 3H), 3.01 (s, 3H), 2.43–2.21 (m, 4H), 2.11–1.87 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.0, 166.8, 152.0, 94.2, 37.0, 36.8, 32.8, 27.8, 18.8. HRMS (ESI) calcd for $C_{10}H_{14}N_2O_3$ $[M+H]^+$ 211.1077, found 211.1072.

6.6.5. 5-*sec*-Butylisoxazol-3-yl dimethylcarbamate (15e)

Prepared using the general procedure above (*Method G*) from 5-*sec*-butylisoxazol-3-ol (**13e**) (45.1 mg, 0.32 mmol), potassium *tert*-butoxide (0.48 mL, 0.48 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.07 mL, 0.80 mmol), and THF (2.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15e** as a clear oil (55.5 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.14 (s, 1H), 3.09 (s, 3H), 3.01 (s, 3H), 2.82 (sextet, J = 7.0 Hz, 1H), 1.72 (doublet of quintet, J = 14.3, 7.2 Hz, 1H), 1.61 (doublet of quintet, J = 14.3, 7.2 Hz, 1H), 1.27 (d, J = 7.0 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz,

$CDCl_3$) δ 179.4, 166.7, 152.0, 94.3, 37.0, 36.8, 34.7, 28.2, 18.1, 11.5. HRMS (ESI) calcd for $C_{10}H_{16}N_2O_3$ $[M+H]^+$ 213.1234, found 213.1243.

6.6.6. 5-Cyclopentylisoxazol-3-yl dimethylcarbamate (15f)

Prepared using the general procedure above (*Method G*) from 5-cyclopentylisoxazol-3-ol (**13f**) (250 mg, 1.63 mmol), potassium *tert*-butoxide (2.12 mL, 2.12 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.37 mL, 4.08 mmol), and THF (10.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15f** as a clear oil (300 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.13 (d, J = 0.8 Hz, 1H), 3.14 (quintet, J = 7.5 Hz, 1H), 3.09 (s, 3H), 3.01 (s, 3H), 2.12–1.97 (m, 2H), 1.80–1.70 (m, 4H), 1.70–1.60 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.9, 166.6, 151.9, 93.9, 38.1, 36.9, 36.6, 31.5, 25.2. HRMS (ESI) calcd for $C_{11}H_{16}N_2O_3$ $[M+Na]^+$ 247.1053, found 247.1036.

6.6.7. 5-(Pentan-2-yl)isoxazol-3-yl dimethylcarbamate (15g)

Prepared using the general procedure above (*Method G*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (170 mg, 1.09 mmol), potassium *tert*-butoxide (1.42 mL, 1.42 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.25 mL, 2.74 mmol), and THF (7.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15g** as a clear oil (207 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.11 (s, 1H), 3.06 (s, 3H), 2.98 (s, 3H), 2.88 (sextet, J = 7.0 Hz, 1H), 1.66 (ddt, J = 13.4, 9.5, 6.5 Hz, 1H), 1.50 (ddt, J = 13.4, 9.5, 6.5 Hz, 1H), 1.38–1.26 (m, 2H), 1.24 (d, J = 7.0 Hz, 3H), 0.87 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.5, 166.6, 151.9, 94.1, 37.4, 36.9, 36.7, 32.9, 20.1, 18.5, 13.9. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+H]^+$ 227.1317, found 227.1677.

6.6.8. 5-(Pentan-3-yl)isoxazol-3-yl dimethylcarbamate (15h)

Prepared using the general procedure above (*Method G*) from 5-(pentan-3-yl)isoxazol-3-ol (**13h**) (73.5 mg, 0.47 mmol), potassium *tert*-butoxide (0.71 mL, 0.71 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.11 mL, 1.18 mmol), and THF (3.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15h** as a clear oil (89.3 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 (s, 1H), 3.10 (s, 3H), 3.01 (s, 3H), 2.67–2.59 (m, 1H), 1.78–1.50 (m, 4H), 0.87 (t, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3, 166.7, 152.0, 95.1, 42.3, 37.0, 36.8, 26.3, 11.7. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+H]^+$ 227.139, found 227.1392.

6.6.9. 5-(Heptan-3-yl)isoxazol-3-yl dimethylcarbamate (15i)

Prepared using the general procedure above (*Method G*) from 5-(heptan-3-yl)isoxazol-3-ol (**13i**) (230 mg, 1.25 mmol), potassium *tert*-butoxide (1.63 mL, 1.63 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.29 mL, 3.13 mmol), and THF (8.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15i** as a clear oil (194 mg, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 (s, 1H), 3.08 (s, 3H), 2.99 (s, 3H), 2.68 (quintet, J = 7.2 Hz, 1H), 1.70–1.53 (m, 4H), 1.34–1.12 (m, 4H), 0.88–0.80 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.5, 166.7, 151.9, 95.0, 40.7, 36.9, 36.7, 33.0, 29.3, 26.7, 22.7, 14.0, 11.6. HRMS (ESI) calcd for $C_{13}H_{22}N_2O_3$ $[M+H]^+$ 255.1427, found 255.1630.

6.6.10. 5-Isobutylisoxazol-3-yl dimethylcarbamate (15j)

Prepared using the general procedure above (*Method G*) from 5-isobutylisoxazol-3-ol (**13j**) (252 mg, 1.78 mmol), potassium *tert*-butoxide (2.32 mL, 2.32 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.41 mL, 4.45 mmol), and THF (10.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15j** as a clear oil (248 mg, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.16 (s, 1H), 3.09 (s, 3H), 3.01 (s, 3H), 2.58 (d, J = 7.1 Hz, 2H), 2.09–1.97 (m, 1H), 0.96 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ174.4, 166.8, 152.0, 96.1, 37.0, 36.8, 36.6, 27.6, 22.4. HRMS (ESI) calcd for $C_{10}H_{16}N_2O_3$ $[M+H]^+$ 213.1509, found 213.1261.

6.6.11. 5-(2-Methylbutyl)isoxazol-3-yl dimethylcarbamate (15k)

Prepared using the general procedure above (*Method G*) from 5-(2-methylbutyl)isoxazol-3-ol (**13k**) (300 mg, 1.93 mmol), potassium *tert*-butoxide (2.5 mL, 2.5 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.44 mL, 4.83 mmol), and THF (10 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15k** as a clear oil (359 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 (s, 1H), 3.09 (s, 3H), 3.00 (s, 3H), 2.69 (dd, J = 15.0, 7.0 Hz, 1H), 2.52 (dd, J = 15.0, 7.0 Hz, 1H), 1.79 (doublet of quintet, J = 13.5, 7.2 Hz, 1H), 1.47–1.32 (m, 1H), 1.27–1.16 (m, 1H), 0.92 (d, J = 6.7 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.5, 166.8, 152.0, 96.2, 37.0, 36.7, 34.6, 33.9, 29.2, 19.2, 11.4. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+Na]^+$ 249.121, found 249.1201.

6.6.12. 5-Neopentylisoxazol-3-yl dimethylcarbamate (15l)

Prepared using the general procedure above (*Method G*) from 5-neopentylisoxazol-3-ol (**13l**) (230 mg, 1.63 mmol), potassium *tert*-butoxide (2.12 mL, 2.12 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.37 mL, 4.07 mmol), and THF (7.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15l** as a clear oil (262 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.13 (s, 1H), 3.04 (s, 3H), 2.95 (s, 3H), 2.54 (s, 2H), 0.93 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.3, 166.5, 151.8, 97.0, 41.5, 36.8, 36.6, 31.6, 29.3. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+NH_4]^+$ 244.1656, found 244.1668.

6.6.13. 5-Isopentylisoxazol-3-yl dimethylcarbamate (15m)

Prepared using the general procedure above (*Method G*) from 5-isopentylisoxazol-3-ol (**13m**) (300 mg, 1.93 mmol), potassium *tert*-butoxide (2.5 mL, 2.5 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.44 mL, 4.83 mmol), and THF (10.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15m** as a clear oil (342 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 (s, 1H), 3.10 (s, 3H), 3.01 (s, 3H), 2.71 (t, J = 8.0, 7.6 Hz, 2H), 1.68–1.54 (m, 3H), 0.93 (d, J = 6.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.4, 166.7, 151.9, 95.1, 36.9, 36.6, 35.9, 27.5, 25.4, 22.2. HRMS (ESI) calcd for $C_{11}H_{18}N_2O_3$ $[M+H]^+$ 227.139, found 227.1398.

6.6.14. 5-(2-Cyclopropylethyl)isoxazol-3-yl dimethylcarbamate (15n)

Prepared using the general procedure above (*Method G*) from 5-(2-cyclopropylethyl)isoxazol-3-ol (**13n**) (80 mg, 0.54 mmol), potassium *tert*-butoxide (1 M in THF, 0.81 mL, 0.81 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.125 mL, 1.35 mmol), and THF (5.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15n** as a clear liquid (90 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.73 (s, 1H), 5.67 (s, 1H), 2.73 (t, J = 7.6 Hz, 2H), 1.56 (q, J = 7.3 Hz, 2H), 0.79–0.63 (m, 1H), 0.54–0.35 (dq, J = 8.5, 4.2 Hz 2H), 0.06 (dt, J = 5.9, 4.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.1, 166.8, 152.0, 95.5, 37.0, 36.8, 32.5, 27.8, 10.6, 4.6. HRMS (ESI) calcd for $C_{11}H_{16}N_2O_3$ $[M+H]^+$ 225.1234, found 225.1229.

6.6.15. 5-(2-Cyclobutylethyl)isoxazol-3-yl dimethylcarbamate (15o)

Prepared using the general procedure above (*Method G*) from 5-(2-cyclobutylethyl)isoxazol-3-ol (**13o**) (50 mg, 0.33 mmol), potassium *tert*-butoxide (1 M in THF, 0.5 mL, 0.5 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.08 mL, 0.83 mmol), and THF (3.5 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15o** as a clear liquid (50 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.12 (s, 1H), 3.08 (s, 3H), 2.99 (s, 3H), 2.59 (t, J = 7.7 Hz, 2H), 2.28 (h, J = 7.7 Hz, 1H), 2.08–1.98 (m, 2H), 1.90–1.78 (m, 2H), 1.75 (q, J = 7.6 Hz, 2H), 1.59 (pd, J = 9.0, 2.5 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.1, 166.6, 151.8, 95.2, 36.8, 36.6, 35.2, 34.1, 27.9, 25.2, 18.3. HRMS (ESI) calcd for $C_{12}H_{18}N_2O_3$ $[M+H]^+$ 239.1390, found 239.1389.

6.6.16. 5-(2-Cyclopentylethyl)isoxazol-3-yl dimethylcarbamate (15p)

Prepared using the general procedure above (*Method G*) from 5-(2-cyclopentylethyl)isoxazol-3-ol (**13p**) (58.4 mg, 0.32 mmol), potassium *tert*-butoxide (0.48 mL, 0.48 mmol), *N*,*N*-dimethylcarbamoyl chloride (0.074 mL, 0.81 mmol), and THF (1.0 mL). Aqueous work up followed by silica gel flash chromatography (20% ethyl acetate in hexane) of the residue yielded **15p** as a clear oil (73.0 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 (s, 1H), 3.09 (s, 3H), 3.01 (s, 3H), 2.71 (t, J = 7.9 Hz, 2H), 1.86–1.73 (m, 3H), 1.69 (q, J = 7.2 Hz, 2H), 1.65–1.57 (m, 2H), 1.56–1.48 (m, 2H), 1.20–0.99 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.5, 166.8, 152.0, 95.3, 39.6, 37.0, 36.8, 33.5, 32.5, 26.9, 25.3. HRMS (ESI) calcd for $C_{13}H_{20}N_2O_3$ $[M+H]^+$ 253.1547, found 253.1525.

6.7. Synthesis of *N*-substituted derivatives of 13g (17g–26g)

6.7.1. 2-(Azetidine-1-carbonyl)-5-(pentan-2-yl)isoxazol-3(2*H*)-one (17g)

To a solution of triphosgene (63.5 mg, 0.21 mmol, 0.35 equiv) in DCM (1.0 mL) was added a solution of 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (94.0 mg, 0.61 mmol, 1 equiv) in DCM (2.0 mL), and the resulting mixture was allowed to stir at room temperature for 5.3 h. To the reaction mixture, a solution of azetidine hydrochloride (85.0 mg, 0.91 mmol, 1.5 equiv) and diisopropylethylamine (0.35 mL, 1.99 mmol, 3.3 equiv) in dichloromethane (3 mL) was added drop wise, and the reaction was stirred for an additional 30 min. The subsequent reaction mixture was washed with aqueous solution of ammonium chloride, and the organic phase was dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography eluting with 20% ethyl acetate in hexanes to yield the desired compound **17g** as clear oil (59.3 mg, 41% yield). Starting material recovered (18.0 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (s, 1H), 4.37 (s, 2H), 4.19 (s, 2H), 2.73 (sextet, J = 7.0 Hz, 1H), 2.36 (quintet, J = 7.8 Hz, 2H), 1.71–1.59 (m, 1H), 1.56–1.45 (m, 1H), 1.43–1.29 (m, 2H), 1.25 (d, J = 7.0 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.06, 165.57, 148.98, 95.50, 53.01, 49.54, 36.63, 33.18, 20.14, 17.84, 16.57, 13.94. HRMS (ESI) calcd for $C_{12}H_{18}N_2O_3$ $[M+H]^+$ 239.139, found 239.1371.

6.7.2. *N*-Ethyl-*N*-methyl-3-oxo-5-(pentan-2-yl)isoxazole-2(3*H*)-carboxamide (18g)

To a solution of triphosgene (66.2 mg, 0.22 mmol, 0.35 equiv) in DCM (1.0 mL) was added a solution of 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (98.0 mg, 0.63 mmol, 1 equiv) in DCM (2.0 mL), and the resulting mixture was allowed to stir at room temperature for 2 h. To the reaction mixture, a solution of *N*-ethylmethyl amine (0.8 mL, 0.95 mmol, 1.5 equiv) and diisopropylethylamine (0.2 mL, 1.14 mmol, 1.8 equiv) in dichloromethane (1.0 mL) was added drop wise, and the reaction was stirred for an additional 30 min. The subsequent reaction mixture was washed with aqueous solution of ammonium chloride, and the organic phase was dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography eluting with 20% ethyl acetate in hexanes to yield the desired compound **18g** as clear oil (66.1 mg, 44% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (s, 1H), 3.47 (br s, 2H), 3.09 (br s, 3H), 2.75 (hextet, J = 7.0 Hz, 1H), 1.72–1.61 (m, 1H), 1.56–1.47 (m, 1H), 1.45–1.31 (m, 2H), 1.26 (d, J = 7.0 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.91, 168.23, 150.03, 94.58, 44.80, 36.68, 35.97, 33.31, 20.14, 17.84, 13.96, 12.24. HRMS (ESI) calcd for $C_{12}H_{20}N_2O_3$ $[M+H]^+$ 241.1547, found 241.1527.

6.7.3. *N*,*N*-Diethyl-3-oxo-5-(pentan-2-yl)isoxazole-2(3*H*)-carboxamide (19g)

Prepared using the general procedure above (*Method F*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (85.0 mg, 0.55 mmol), *N*,*N*-diethylcarbamoyl chloride (0.35 mL, 2.74 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **19g** as a clear oil (96.0 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (s, 1H), 3.49 (q, J = 6.5 Hz, 4H), 2.74 (hextet, J = 7.0 Hz, 1H), 1.74–1.61 (m, 1H), 1.56–1.45 (m, 1H), 1.45–1.29 (m, 2H), 1.27–1.21 (m, 9H), 0.91 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.81, 168.36, 149.62, 94.31, 42.47, 36.45, 33.07, 19.89, 17.59, 13.71, 13.30. Due to signal broadening (slow chemical exchange) in carboxamide the two CH_2 and two CH_3 of the *N*,*N*-diethyl overlap in ^{13}C NMR. This is consistent with similar observations in seen ^{1}H NMR (see Fig. 2 for explanation). HRMS (ESI) calcd for $C_{13}H_{22}N_2O_3$ $[M+H]^+$ 255.1703, found 255.1687.

6.7.4. 5-(Pentan-2-yl)-2-(pyrrolidine-1-carbonyl)isoxazol-3(2*H*)-one (20g)

Prepared using the general procedure above (*Method F*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (65.1 mg, 0.42 mmol), 1-pyrrolidinecarbonyl chloride (0.23 mL, 2.10 mmol), and toluene (2.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **20g** as a clear oil (38.7 mg, 37%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (s, 1H), 3.73 (t, J = 6.3 Hz, 2H), 3.57 (t, J = 6.4 Hz, 2H), 2.75 (sextet, J = 7.0 Hz, 1H), 1.94 (s, 4H), 1.72–1.61 (m, 1H), 1.56–1.47 (m, 1H), 1.45–1.31 (m, 2H), 1.26 (d, J = 7.0 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.14, 167.78, 148.43, 94.91, 48.37, 47.31, 36.68, 33.24, 25.89, 24.48, 20.14, 17.83, 13.96. HRMS (ESI) calcd for $C_{13}H_{20}N_2O_3$ $[M+H]^+$ 253.1547, found 253.1547.

6.7.5. 2-(Morpholine-4-carbonyl)-5-(pentan-2-yl)isoxazol-3(2*H*)-one (21g)

Prepared using the general procedure above (*Method F*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (99.4 mg, 0.64 mmol), 4-morpholinecarbamoyl chloride (0.37 mL, 3.20 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **21g** as a clear oil (133 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (s, 1H), 3.80–3.75 (m, 4H), 3.64–3.56 (m, 4H), 2.77 (sextet, J = 7.0 Hz, 1H), 1.73–1.61 (m, 1H), 1.57–1.48 (m, 1H), 1.45–1.30 (m, 2H), 1.27 (d, J = 7.0 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 183.23, 167.55, 148.92, 94.29, 66.66, 47.73, 45.19, 36.53, 33.22, 20.00, 17.68, 13.80. HRMS (ESI) calcd for $C_{13}H_{20}N_2O_4$ $[M+H]^+$ 269.1496, found 269.1476.

6.7.6. 5-(Pentan-2-yl)isoxazol-3-yl ethyl(methyl)carbamate (23g)

To a solution of triphosgene (66.2 mg, 0.22 mmol, 0.35 equiv) in DCM (1.0 mL) was added a solution of 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (98.0 mg, 0.63 mmol, 1 equiv) in DCM (2.0 mL), and the resulting mixture was allowed to stir at room temperature for 2 h. To the reaction mixture, a solution of *N*-ethylmethyl amine (0.8 mL, 0.95 mmol, 1.5 equiv) and diisopropylethylamine (0.2 mL, 1.14 mmol, 1.8 equiv) in dichloromethane (1.0 mL) was added drop wise, and the reaction was stirred for an additional 30 min. The subsequent reaction mixture was washed with aqueous solution of ammonium chloride, and the organic phase was dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography eluting with 20% ethyl acetate in hexanes to yield the desired compound **23g** as clear oil (30.0 mg, 20% yield). (*Observed (Z)- and (E)-amide rotamers in 1:1 ratio, the following integral ratios correspond to the 1:1 mixture*) ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.14 (s, 2H, both (*Z*)- and (*E*)-), 3.46 (q, J = 7.2 Hz, 2H), 3.39 (q, J = 7.2 Hz, 2H), 3.05 (s, 3H), 2.98 (s, 3H), 2.90 (sextet, J = 7.0 Hz, 2H), 1.74–1.62 (m, 2H), 1.58–1.46 (m, 2H), 1.39–1.29 (m, 4H), 1.27 (d, J = 7.0 Hz, 6H), 1.21 (t, J = 7.2 Hz, 3H), 1.18 (t, J = 7.2 Hz, 3H), 0.89 (t, J = 7.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.57, 166.75, 166.72, 151.71, 151.57, 94.21, 94.16, 44.59, 44.45, 37.47, 34.51, 34.18, 32.98, 20.21, 18.62, 14.02, 13.28, 12.37. HRMS (ESI) calcd for $C_{12}H_{20}N_2O_3$ $[M+H]^+$ 241.1547, found 241.1532.

6.7.7. 5-(Pentan-2-yl)isoxazol-3-yl diethylcarbamate (24g)

Prepared using the general procedure above (*Method F*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (85.0 mg, 0.55 mmol), *N*,*N*-diethylcarbamoyl chloride (0.35 mL, 2.74 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **24g** as a clear oil (23.0 mg, 16%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 (s, 1H), 3.43 (q, J = 7.1 Hz, 2H), 3.37 (q, J = 7.2 Hz, 2H), 2.91 (sextet, J = 7.0 Hz, 1H), 1.69 (ddt, J = 13.6, 9.2, 6.5 Hz, 1H), 1.59–1.47 (m, 1H), 1.41–1.28 (m, 2H), 1.27 (d, J = 7.0 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H), 1.20 (t, J = 7.2 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.52, 166.75, 151.39, 94.21, 42.70, 42.34, 37.48, 32.98, 20.22, 18.64, 14.24, 14.02, 13.24. HRMS (ESI) calcd for $C_{13}H_{22}N_2O_3$ $[M+H]^+$ 255.1703, found 255.1687.

6.7.8. 5-(Pentan-2-yl)isoxazol-3-yl pyrrolidine-1-carboxylate (25g)

Prepared using the general procedure above (*Method F*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (65.1 mg, 0.42 mmol), 1-pyrrolidinecarbonyl chloride (0.23 mL, 2.10 mmol), and toluene (2.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **25g** as a clear oil (60.4 mg, 57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 (s, 1H), 3.56 (t, J = 6.6 Hz, 2H), 3.46 (t, J = 6.6 Hz, 2H), 2.90 (sextet, J = 7.0 Hz, 1H), 2.01–1.84 (m, 4H), 1.74–1.63 (m, 1H), 1.58–1.47 (m, 1H), 1.41–1.28 (m, 2H), 1.26 (d, J = 7.0 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.51, 166.67, 150.20, 94.11, 46.80, 46.71, 37.46, 32.97, 25.84, 25.01, 20.21, 18.62, 14.02. HRMS (ESI) calcd for $C_{13}H_{20}N_2O_3$ $[M+H]^+$ 253.1547, found 253.1528.

6.7.9. 5-(Pentan-2-yl)isoxazol-3-yl morpholine-4-carboxylate (26g)

Prepared using the general procedure above (*Method F*) from 5-(pentan-2-yl)isoxazol-3-ol (**13g**) (99.4 mg, 0.64 mmol), 4-morpholinecarbamoyl chloride (0.37 mL, 3.20 mmol), and toluene (3.0 mL). Concentration of the reaction mixture in vacuo followed by silica gel flash chromatography (20–30% ethyl acetate in hexane) yielded **26g** as a clear oil (20.0 mg, 12%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.14 (s, 1H), 3.77–3.69 (m, 4H), 3.68–3.64 (m, 2H), 3.59–3.51 (m, 2H), 2.91 (h, J = 7.0 Hz, 1H), 1.74–1.64 (m, 1H), 1.58–1.48 (m, 1H), 1.39–1.29 (m, 2H), 1.28 (d, J = 7.0 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.86, 166.47, 150.75, 94.11, 66.59, 66.48, 45.20, 44.42, 37.46, 33.01, 20.22, 18.61, 14.03. HRMS (ESI) calcd for $C_{13}H_{20}N_2O_4$ $[M+H]^+$ 269.1496, found 269.1478.

6.8. General procedure for the synthesis of *N*-methyl-3-oxoisoxazole-2(3*H*)-carboxamide

6.8.1. 5-Isobutyl-*N*-methyl-3-oxoisoxazole-2(3*H*)-carboxamide (16j)

To a dry round bottom flask was added 5-isobutylisoxazol-3-ol (**13j**) (165 mg, 1.17 mmol) and dry tetrahydrofuran (7.0 mL) prior to cooling it to 0 °C. To this solution was added potassium *tert*-butoxide (1 M in THF, 1.52 mL, 1.52 mmol) at 0 °C and subsequently the reaction mixture was stirred for 20 min at RT. To this solution was added *N*-methylcarbamoyl chloride (273 mg, 2.92 mmol) and the resulting solution was stirred at room temperature for additional 16 h. The solvent was concentrated and the residue was taken up in dichloromethane and washed with 0.25 M HCl hydrochloric acid, and then brine, and dried over sodium sulfate. The solvent was evaporated and the residue was purified using silica gel column chromatography (20% ethyl acetate in hexane) to yield **16j** as a white semi-solid (207 mg, 90%). ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.83 (s, 1H), 5.56 (s, 1H), 2.96 (d, J = 4.8 Hz, 3H), 2.49 (d, J = 7.2 Hz, 2H), 2.14–1.98 (m, 1H), 0.99 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 174.44, 165.47, 148.45, 98.12, 36.55, 27.16, 26.48, 22.35. HRMS (ESI) calcd for $C_9H_{14}N_2O_3$ $[M+H]^+$ 199.1077, found 199.1086.

6.9. Bioassays

6.9.1. Enzyme inhibition assays

Recombinant WT and G119S *Ag*AChE were prepared as previously described.[12] Recombinant *h*AChE (C1682) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Inhibition potency of carbamate and carboxamide insecticides was assessed by measuring apparent second-order rate constants k_i (mM^{-1} min^{-1}) for inactivation of the enzymes. We followed our previous progressive inactivation approach,[8,12] in which enzymes were incubated with different concentrations of carbamates or carboxamides for differing times before measuring enzyme residual activity (v/v_0). Enzymes were incubated with typically five concentrations of inhibitors (and an inhibitor-free control) for up to 6 min at approximately 1 min intervals. Each concentration was present in the microplate in duplicate, and each experiment was repeated. Note that for the G119S enzyme inhibition was typically very low after 10 min at 10 μM, and so incubations with these enzymes were often extended to 60 min (10 min intervals). Residual activities v/v_0 are the ratio of the rate in the presence of inhibitor to a time-matched inhibitor-free control and thus correct for slow thermal inactivation of the enzyme. At each inhibitor concentration [*I*], plots of $\ln(v/v_0)$ versus time (t) were constructed; the slopes of these unconstrained linear fits represent the pseudo first-order rate constants (k_{obs}) associated with those inhibitor concentrations (see Supplementary material, Fig. S2). These k_{obs} values (obtained in at least two separate experiments) were then plotted against [*I*]; in each case the slope of the unconstrained linear fit is the apparent second-order rate constant k_i (mM^{-1} min^{-1}) for inactivation (see Supplementary material, Fig. S3). Note that the error in k_{obs} is typically smaller than the symbol in these plots (see Fig. S3). The error in k_i is estimated as the standard error in the slope of this plot. Note that inhibitor concentrations [*I*] were chosen to be low enough to remain in the domain where a plot of k_{obs} versus [*I*] was linear. For fast-inactivating carbamates, such as

terbam (**4**) on r*Ag*AChE-WT, we saw signs of saturation behavior above [*I*] = 0.2 μM. Saturation behavior is expected for a two-step mechanism of inhibition.[35] Enzymatic sensitivity ratios (SR) and *Ag*/*h* selectivity for inhibition (Tables 3 and 4) were calculated from the measured k_i values; the error in these ratios was determined using a standard propagation of error method.[20]

6.9.2. Mosquito contact toxicity determination

Anopheles gambiae eggs (G3 (MRA-112) and Akron (MRA-913)) were obtained from MR4, and reared in tap water with fish food for larval sustenance (Tetra Fish, Blacksburg, VA, USA). Adult female non-blood fed *An. gambiae* (both G3 and Akron strains) 3–5 days old, were used for filter paper assays of tarsal contact toxicity, which were performed in exposure tubes according to the 2006 World Health Organization recommendations[18] with slight modification. In brief, filter papers (15 × 12 cm) were treated with 2.0 mL of various concentrations of the carbamate in ethanol, and allowed to dry overnight. For the G3 strain, batches of 20–25 mosquitoes (in triplicate) were transferred to a holding tube and allowed to adapt for one hour. Due to lower colony numbers, toxicity assays with the Akron strain used batches of 10–15 mosquitoes in duplicate. Mosquitoes were then transferred to the exposure tube (held horizontally) that contained a treated filter paper. Knockdown was noted after 1 h, and all mosquitoes were transferred back to the holding tube (held upright), and given free access to 10% (w/v) sugar water. Mortality was recorded at 24 h. Both during exposure and the 23 h period following, mosquito tubes were kept in an environmental chamber at 24 ± 1 °C and 75% RH. To determine LC_{50} values, typically 5–8 concentrations were examined, and mortality data were used for probit analysis using PoloPlus[36] or SAS Probit.

6.10. Computational modeling

Flexible ligand docking of the dimethylcarboxamide tetrahedral covalent intermediate adduct derived from **14c** and mAChE was performed in ICM using default settings for 'covalent' docking mode (ICM-Docking module, Molsoft).[24,25] The receptor structure was prepared for docking starting from PDB entry 2H9Y following the standard conversion procedure in ICM. Two possible stereoisomers of the covalent adduct were sampled and lowest-score solution selected. To get more accurate covalent geometry of the bound ligand, the lowest-scoring ligand pose from docking was subjected to unrestrained minimization of the ligand in fixed explicit receptor (3000 steps) with MMFF94 force-field.[37]

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Supplementary data

Supplementary data (enzyme alignments, details of X-ray crystallography, and calculation of τ values) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.01.026. These data include MOL files and InChiKeys of the most important compounds described in this article.

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