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Targeting Carnitine Biosynthesis: Discovery of New Inhibitors against γ-Butyrobetaine Hydroxylase

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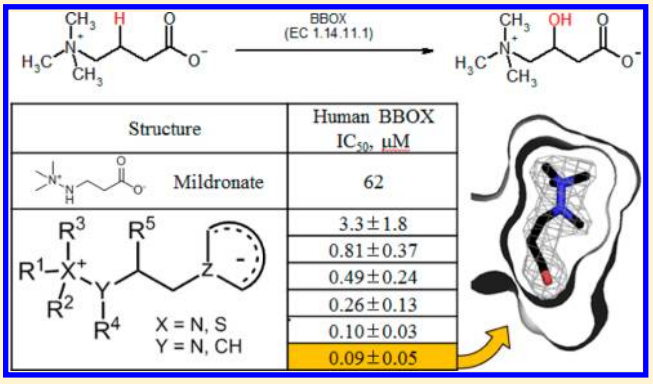
Supporting Information

ABSTRACT: γ-Butyrobetaine hydroxylase (BBOX) catalyzes the conversion of gamma butyrobetaine (GBB) to L-carnitine, which is involved in the generation of metabolic energy from long-chain fatty acids. BBOX inhibitor 3-(1,1,1-trimethylhydrazin-1-ium-2-yl)propanoate (mildronate), which is an approved, clinically used cardioprotective drug, is a relatively poor BBOX inhibitor and requires high daily doses. In this paper we describe the design, synthesis, and properties of 51 compounds, which include both GBB and mildronate analogues. We have discovered novel BBOX inhibitors with improved IC_{50} values; the best examples are in the nanomolar range and about 2 orders of magnitude better when compared to mildronate. For six inhibitors, crystal structures in complex with BBOX have been solved to explain their activities and pave the way for further inhibitor design.



INTRODUCTION

L-Carnitine is an important molecule for the regulation of cellular energy metabolism of fatty acids and glucose. L-Carnitine is involved in long-chain fatty acid transport across the inner membrane of mitochondria, and it facilitates the transport of chain-shortened acyl groups produced in peroxisomes to mitochondria for further energy production.[1] L-Carnitine also takes part in the regulation of glucose metabolism via modulation of free coenzyme A/acetyl-coenzyme A ratio.[2] It is possible to shift the source of metabolic energy production from fatty acid β-oxidation to glucose oxidation by mildly reducing the amount of bioavailable L-carnitine;[3,4] the survival of cardiac muscle cells under ischemic (low-oxygen) conditions is improved because aerobic glucose oxidation consumes less oxygen than fatty acid oxidation.[5]

γ-Butyrobetaine dioxygenase (BBOX), also known as γ-butyrobetaine hydroxylase (GBBH), EC 1.14.11.1, is an enzyme that catalyzes the formation of L-carnitine from 4-(trimethylammonio)butanoate, which is commonly known as γ-butyrobetaine (GBB). Presently, 3-(1,1,1-trimethylhydrazin-1-ium-2-yl)propanoate (mildronate, meldonium, THP), which is structurally similar to the endogenous BBOX substrate, GBB (with an NH group replacing the CH_2 of GBB at the C-4 position), is the only inhibitor of BBOX utilized pharmacologically. Mildronate induces cardioprotective effects by reducing the concentration of L-carnitine.[6,7] Although mildronate is successful in the clinic,[8] high dosages (1 g/day) are required, at least partly due to comparably inefficient inhibition of BBOX (K_i = 16 μM[9]). A recent study has linked the amount of plasma L-carnitine with levels of its metabolite, trimethylamine N-oxide, and the increased risk of cardiovascular and diabetic complications.[10,11] To investigate new L-carnitine-lowering agents, a mildronate-like drug with improved properties must be developed. Recently published three-dimensional structures of BBOX facilitate rational ligand design. Crystal structures of BBOX complexed with GBB and mildronate[12] suggest that both compounds bind to BBOX in a similar manner (Figure 1A). GBB and mildronate make a number of polar and hydrophobic interactions with the enzyme (Figure 1B). A trimethylammonium group is involved in cation−π interactions with nearby aromatic residues; therefore, a positive charge at one end of the ligand is crucial for binding. The carboxyl groups on GBB and mildronate interact with nearby asparagines. Mildronate may form a weak hydrogen bond where GBB does not, between Tyr366 and a secondary nitrogen; however, the observed 3.3 Å distance between the hydrogen acceptor and donor might be too large to facilitate this interaction. The crystal structure of BBOX without ligands[13] revealed that loops covering the catalytic site are disordered and presumably close only upon substrate binding. We describe our efforts toward improved inhibitors of BBOX using chemistry, in vitro binding assays, and structural biology.

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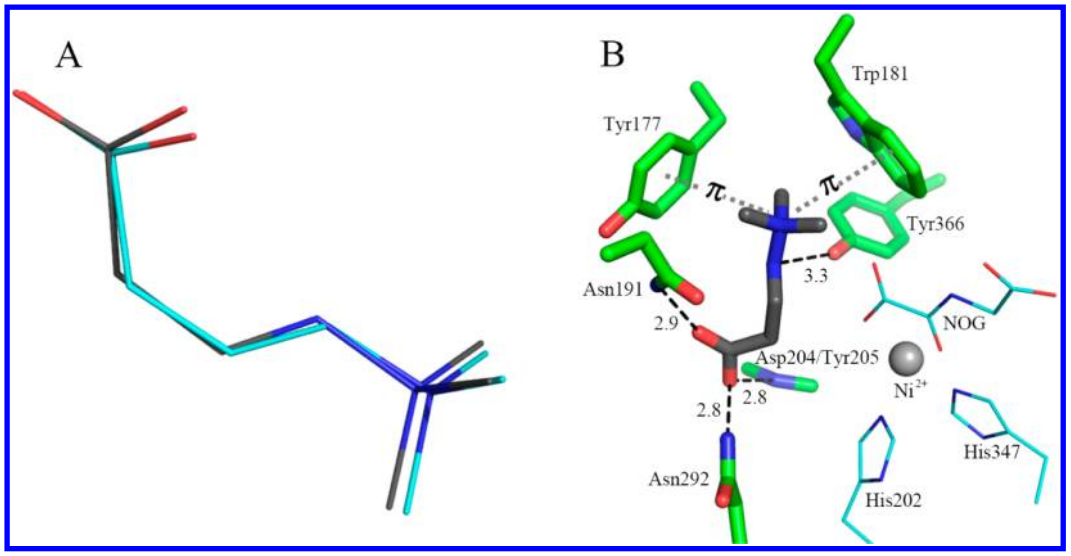

Figure 1. Interactions of GBB and mildronate with BBOX. (A) In crystal structures of BBOX complexes (PDB codes 3O2G and 3MS5[12]), mildronate (dark gray carbons) and GBB (light gray carbons) are bound in nearly identical conformations. (B) Polar interactions of mildronate with BBOX. Hydrogen bonds and distances among interacting atoms are shown. Positively charged trimethylammonium group interacts (cation−π) with nearby aromatic groups. Two metal-coordinating histidines and an analogue of α-ketoglutarate (*N*-oxalylglycine, NOG) are shown for reference as thinner stick models, and metal ion as a sphere.

RESULTS AND DISCUSSION

Shape of the Active Site and Possibilities for Extension of Inhibitors. In Figure 2A, the GBB-binding pocket of BBOX and bound mildronate are shown as accessible surface models. Surface complementarity reveals several regions where inhibitor modification/extension is possible. First, there is an empty cavity around the methyl trimethylammonium group (denoted cavity A). Second, there is an unexploited cavity around the core region of the inhibitor (denoted cavity B). Third, there is a substrate entry tunnel on the side of the core region opposing cavity B (denoted cavity C). Fourth, there is space near the inhibitor's carboxyl group (denoted cavity D) occupied by two water molecules, which could be exchanged for the polar groups of a new inhibitor. Finally, because the loops surrounding the active site in the unliganded form of BBOX are disordered,[13] different inhibitors might influence the shape of the binding pocket.

The substrate entry channel (cavity C) is relatively narrow, and extending the ligand in this direction would likely interact unfavorably with the protein. Replacing the water molecules in cavity D with an extended ligand is problematic due to a total requirement of an acid residue in the inhibitor. We have mainly focused on inhibitor extension in cavities A and B and carboxylate replacement by other acidic groups.

Chemistry. Two groups of possible BBOX ligands were synthesized and tested for inhibition potency (Table 1). The first group of compounds includes GBB derivatives with chemical modifications altering the trimethylammonium, carboxylate, and/or butanoic acid core structure (Table 1, entries 1−37). A hydrazinium functionality is present in every member of the second group of compounds (Table 1, entries 38−52); this group can be regarded as 3-(1,1,1-trimethylhydrazin-1-ium-2-yl)propanoate (mildronate) analogues.

Synthetic strategies for preparation of GBB analogues are summarized in Schemes 1−11, and the routes toward mildronate analogues are shown in Schemes 12−16.

Convenient modifications of the ammonium portion of the GBB analogues utilize commercially available ethyl 4-bromobutanoate (**1**) (Scheme 1). Straightforward amination of the bromo derivative **1** with commercially available *N*,*N*-dimethylethylamine, *N*,*N*-diethylmethylamine, or 2-(dimethylamino)-ethanol afforded quaternary ammonium ester intermediates **2**−**4**, respectively, which by passage through a column with anion-exchange resin Amberlite IRA-410 (OH form) were hydrolyzed into betaines **14**−**16**. Although this short strategy in efficiency could seem to be a method of choice for the preparation of quaternary nitrogen structures, the approach is limited by commercial availability or ease of preparation of the necessary tertiary amines for this conversion. Similar treatment of bromo derivative **1** with *N*-methylcyclopropanamine (**30a**), *N*-methylcyclobutanamine (**30b**), *N*-methylcyclopentanamine (**30c**) (note: the preparation of amines **30a**−**c** is shown in Scheme 2), and dimethylamine afforded the corresponding tertiary 4-amino derivatives **5a**−**d**. The resulting aminobutanoates **5a**−**c** were converted into quaternized ammonium salts **6**−**13** by treatment with either iodomethane (**8**−**10**) or other appropriate alkyl halide R^2X (**6**, **7**, **11**, and **12**). Treatment of aminobutanoate **5d** with 2-fluoroethyl trifluoromethanesulfonate, followed by exchange of the trifluoromethanesulfonate ion to chloride with anion-exchange resin Amberlite IRA-410 (Cl form), yielded **13.** The intermediate esters **6**−**12** were hydrolyzed into betaines **17**−**23**, respectively, by Amberlite IRA-410 (OH form), and 2-fluoroethylammonium derivative **13** was converted into carboxylic acid **24** by treatment with aqueous HCl (Scheme 1).

Alicyclic methylamines **30a**−**c**, necessary for the synthesis of aminobutanoates **5a**−**c**, were prepared as hydrochlorides by Boc-protection of the primary alicyclic amines **27a**−**c** followed by alkylation of the obtained carbamates **28a**−**c** with iodomethane into methylderivatives **29a**−**c**, and acidic removal of the Boc-protecting group of the latter by HCl methanol solution (Scheme 2).

GBB analogues **44**−**54** were prepared starting from commercially available 4-aminobutanoic acid (**31**) as shown in Scheme 3. Reductive amination of commercial 4-aminobutanoic acid (**31**) by HCHO/HCOOH procedure gave 3-carboxy-*N*,*N*-dimethyl-1-propanaminium chloride[14] (**32**), which was transformed into methyl ester hydrochloride **33a** with $SOCl_2$/MeOH.

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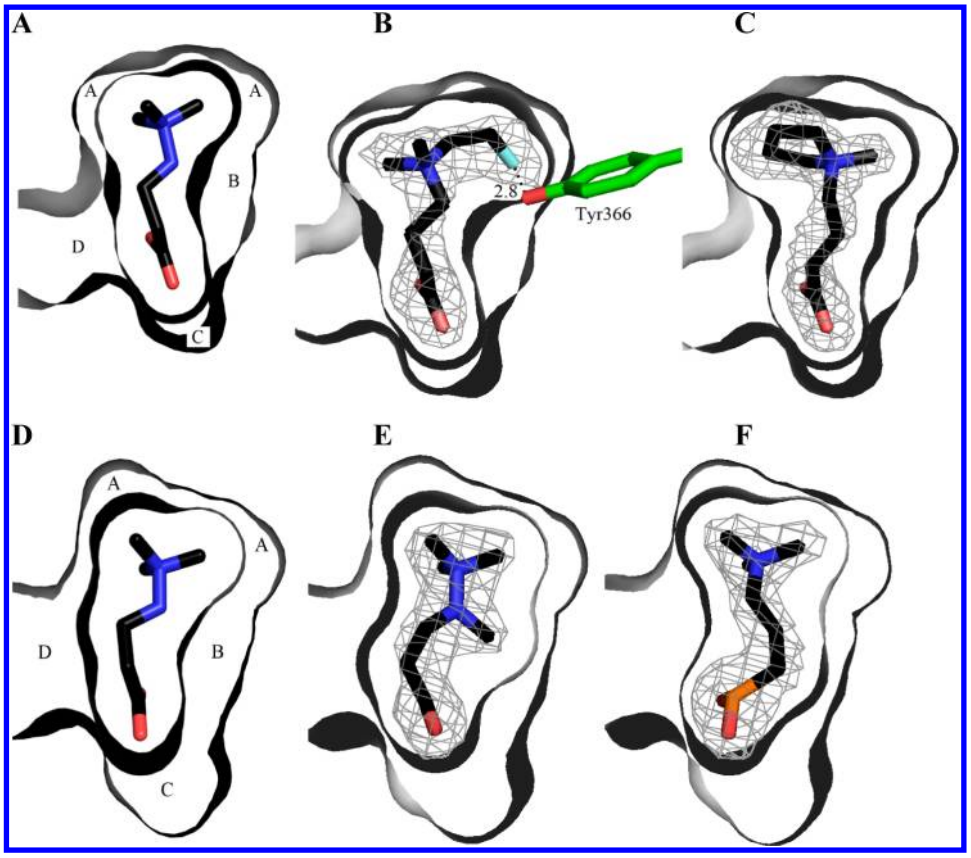

Figure 2. Comparison of binding modes of mildronate and other newly synthetized compounds. Cross sections of the substrate binding pocket's accessible surface (outer contour) and accessible surface of the bound inhibitor (assuming that protein is absent, inner contour) are shown. In panels A and D, four cavities (A–D) for potential expansion of a mildronate-based inhibitor are shown. Cavity A can be filled by the cyclization of two methyl moieties (panel C, **26**) or by an extension of the methyl group of the trimethylammonium moiety and an additional H-bond (panel B, **24**). Cavity B is partially filled by **98** via an additional methyl group (panel E) and by **78** via a different backbone conformation due to substitution of the carboxylate group with a phosphinate group (panel F). Mildronate is shown twice (panels A and D) due to slightly different views in the top and bottom rows. The $F_o - F_c$ omit electron density maps are shown for all new inhibitors (contoured at 3σ).

Direct treatment of the ester **33a** with 1-bromopropane in the presence of K_2CO_3 or allylbromide/$NaHCO_3$ yielded quaternized structures **34** and **35**, respectively, in a one-pot procedure. Alternatively, hydrochloride **33a** was deprotonated with potassium carbonate and the obtained crude product was purified by vacuum distillation to afford dimethylaminobutanoate as free base **33b**. The obtained amine **33b** was alkylated with appropriate alkyl halides RX to form quaternized structures **36**–**43**. Obtained ammonium esters **34**–**42** were converted into betaines **44**–**52** by passage through a column with ion-exchange resin Amberlite IRA-410 (OH form), but esters **42** and **43** were hydrolyzed to carboxylic acids **53** and **54** respectively by treatment with aqueous HCl. It is noteworthy that 2-halogenethyl derivatives **42** and **43** from the ion-exchange resin treatment formed vinyl derivative **52**; thus the formation of **53** and **54** was possible under acidic conditions only.

Essentially the same synthetic sequence as Scheme 3 was employed for preparation of GBB analogue **59** containing a cyclohexane moiety (Scheme 4). As a starting material for this synthesis, we used commercially available 3-aminocyclohexanecarboxylic acid (**55**) (in a form of racemic cis and trans isomer mixture).

The GBB analogue **64**, containing an extra methyl group in a butanoic acid backbone (Scheme 5), was prepared by treatment of commercial racemic γ-valerolactone (**60**) with iodotrimethylsilane to give 4-iodopentanoic acid (**61**),[15] which was esterified with HCl/MeOH. The obtained methyl ester **62** entered a highly reluctant S_N2 reaction with trimethylamine, yielding 4-trimethylammonium derivative **63**. Elimination of HI was the dominant side reaction lowering the yield of **63**. The necessary betaine **64** was obtained by passing a solution of the ester **63** through a column with ion-exchange resin Amberlite IRA-410 (OH form).

The known[16] unsaturated GBB analogue **68** was prepared from ethyl 4-bromocrotonate (**66**) by treatment of the latter with trimethylamine, followed by ester hydrolysis of the obtained trimethylammonium derivative **67** with Amberlite IRA 410 (OH form) (Scheme 6).

Sulfonate analogues **70** and **71** of GBB were prepared by aminolysis of 1,3-propanesultone (**69**)[17] with trimethylamine and dimethylethylamine, respectively (Scheme 7).

To prepare the phosphonic acid analogue of GBB, **74** (Scheme 8), commercial diethyl (3-bromopropyl)phosphonate (**72**) was treated with trimethylamine to give trimethylammonium derivative **73**. Passing a solution of **73** through a column with ion-exchange resin Amberlyst A-21 (Cl form), followed by treatment with concentrated HCl at reflux temperature, allowed us to obtain target phosphonic acid **74**.

The synthesis of phospinic acid analogues **78** and **79** of GBB was performed as outlined in Scheme 9. Thus, readily available[18] ethyl (diethoxymethyl)phosphinate (**75**) was treated with NaH followed by 1,3-dibromopropane to afford 3-bromopropyl

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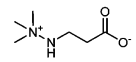
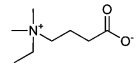
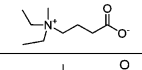
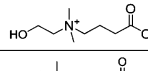
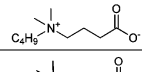
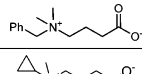
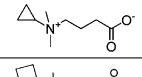
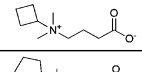
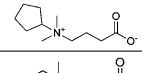
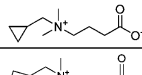
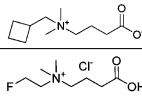
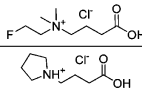
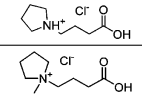
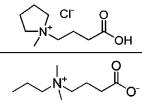
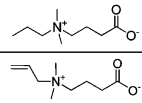
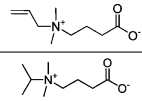
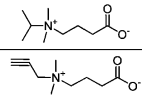
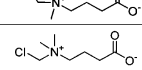
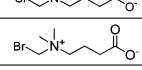
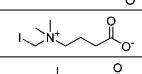
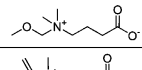
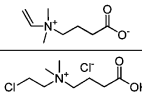
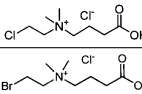
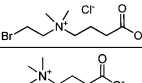
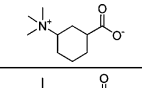
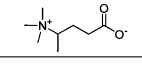
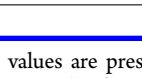
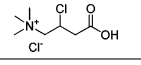
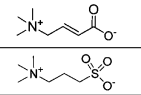
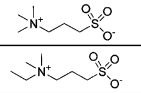
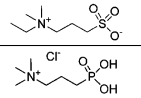
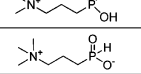
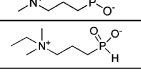
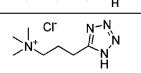
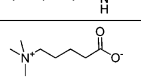
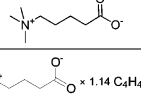
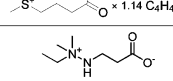
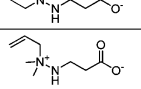
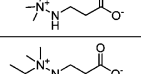
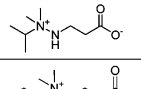
Table 1. Prepared GBB and Mildronate Analogues: Synthetic Sequences and IC_{50} Values[a]

Entry	No.	Structure	Scheme no.: synthesis sequence or reference in literature	Human BBOX IC_{50}, μM
Mildronate			9	62 (Tars et al., 2010)
1	**14**		1: **2**, **14**	3.3±1.8
2	**15**		1: **3**, **15**	100±36
3	**16**		1: **4**, **16**	1000
4	**17**		1: **5d**, **6**, **17**	>1000
5	**18**		1: **5d**, **7**, **18**	>1000
6	**19**		1: **5a**, **8**, **19**	2.1±0.7
7	**20**		1: **5b**, **9**, **20**	0.81±0.37
8	**21**		1: **5c**, **10**, **21**	>1000
9	**22**		1: **5d**, **11**, **22**	>1000
10	**23**		1: **5d**, **12**, **23**	>1000
11	**24**		1: **5d**, **13**, **24**	0.61±0.22
12	**25**		33	1000
13	**26**		33	0.49±0.24
14	**44**		3: **34**, **44**	>1000
15	**45**		3: **35**, **45**	2.7±0.8
16	**46**		3: **36**, **46**	5.7±1.6
17	**47**		3: **37**, **47**	1.4±0.6
18	**48**		3: **38**, **48**	268±54
19	**49**		3: **39**, **49**	>1000
20	**50**		3: **40**, **50**	1000
21	**51**		3: **41**, **51**	>1000
22	**52**		3: **42**, **52**	127±32
23	**53**		3: **42**, **53**	0.26±0.13
24	**54**		3: **43**, **54**	1.2±0.3
25	**59**		4: **56**, **57**, **58**, **59**	>1000
26	**64**		5: **61**, **62**, **63**, **64**	343±117
27	**65**		39	459±66
28	**68**		6: **66**, **67**, **68**	3.8±1.8
29	**70**		7: **69**, **70**	1.7±0.4
30	**71**		7: **69**, **71**	6.7±1.2
31	**74**		8: **72**, **73**, **74**	0.10±0.03
32	**78**		9: **75**, **76**, **77a**, **78**	0.52±0.35
33	**79**		9: **75**, **76**, **77b**, **79**	0.53±0.17
34	**82**		10: **80**, **81**, **82**	>1000
35	**85**		11: **83**, **84**, **85**	>1000
36	**86**		41	388±32
37	**91**		12: **87**, **88a**, **91**	140±52
38	**92**		12: **87**, **88b**, **92**	119±43
39	**93**		12: **87**, **88c**, **93**	>1000
40	**94**		12: **87**, **88d**, **94**	1000
41	**95**		12: **87**, **88e**, **95**	6.8±1.7
42	**96**		12: **87**, **88e**, **96**	10.2±4.4
43	**97**		12: **87**, **88f**, **97**	24±8
44	**98**		12: **89a**, **90a**, **98**	0.09±0.05
45	**99**		12: **89a**, **90b**, **99**	7.2±2.7
46	**100**		12: **89a**, **90c**, **100**	3.1±1.8
47	**101**		12: **87**, **89b**, **90d**, **101**	2.8±1.9
48	**104**		13: **102**, **103**, **104**	>1000
49	**106**		14: **87**, **105**, **106**	>1000
50	**110**		15: **107**, **108**, **109**, **110**	87±33
51	**114**		16: **111**, **112**, **113**, **114**	117±21

[a]Apparent IC_{50} values are presented as mean ± standard deviation of three independent experiments (for compounds with IC_{50} values less than 1000 μM). A potential effect of inhibitors themselves being consumed during the reaction is not taken into account. The IC_{50} curves for 23 compounds with IC_{50} value lower than that of mildronate (below 62 μM) are represented in the Supporting Information (Figure S1).

phosphinate **76**. The phosphinate **76** was condensed with trimethylamine or dimethylethylamine to form the corresponding ammonium bromides **77a** and **77b**, which were further heated with concentrated HCl at reflux temperature and passed through

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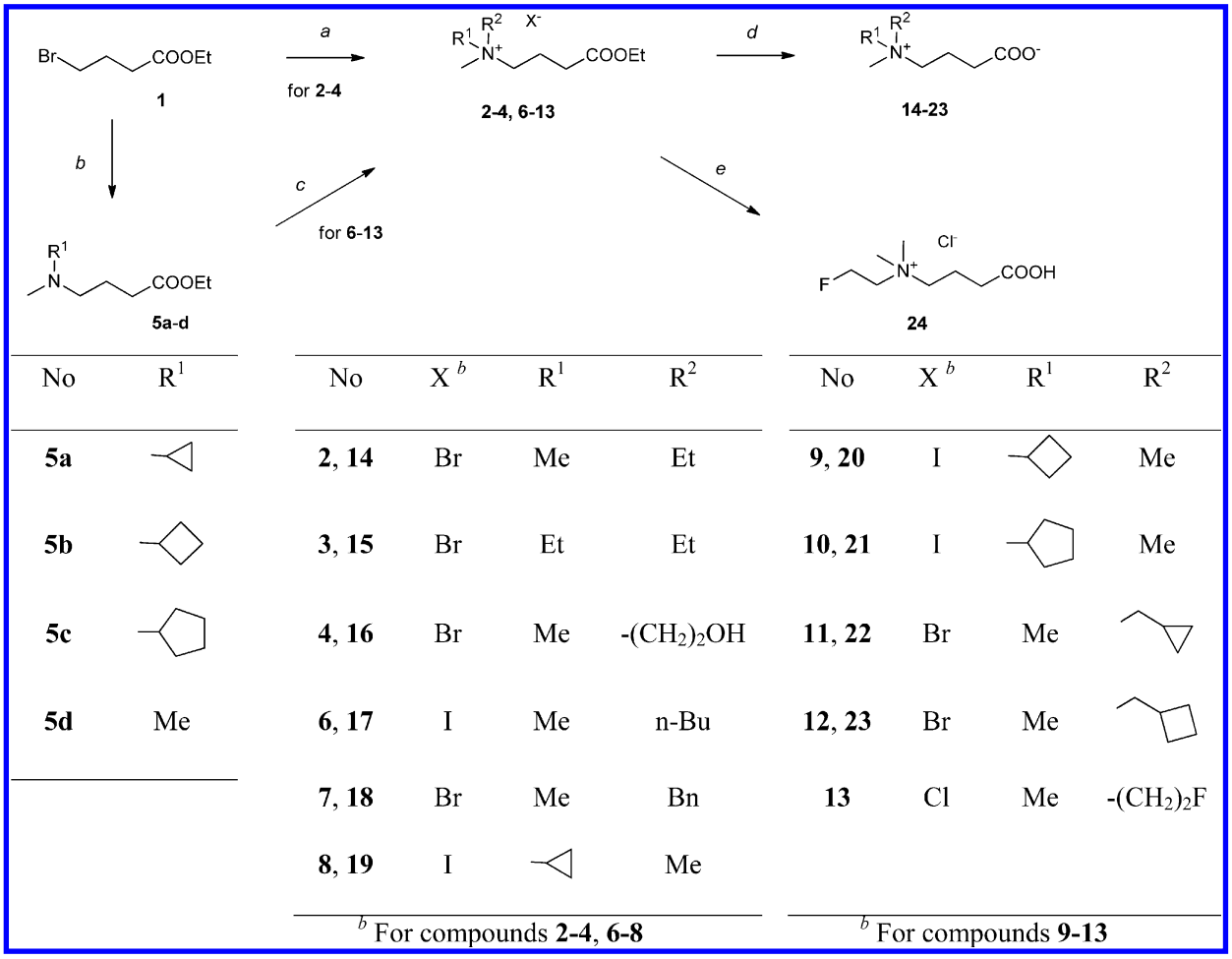
Scheme 1. Synthesis of GBB Analogues 14−24 from Ethyl 4-Bromobutanoate (1)[a]



No	R^1
5a	
5b	
5c	
5d	Me

No	X [b]	R^1	R^2
2, 14	Br	Me	Et
3, 15	Br	Et	Et
4, 16	Br	Me	-$(CH_2)_2$OH
6, 17	I	Me	n-Bu
7, 18	Br	Me	Bn
8, 19	I		Me

[b] For compounds **2-4**, **6-8**

No	X [b]	R^1	R^2
9, 20	I		Me
10, 21	I		Me
11, 22	Br	Me	
12, 23	Br	Me	
13	Cl	Me	-$(CH_2)_2$F

[b] For compounds **9-13**

[a]Reagents and conditions: (a) For **2**, Me_2NEt, CH_2Cl_2, rt, yield 94%; for **3**, $MeNEt_2$, acetone, rt, yield 87%; for **4**, $Me_2N(CH_2)_2OH$, CH_2Cl_2, rt, yield 52%. (b) For **5a−c**, MeR^1NH (0.67 equiv), TEA, CH_3CN, rt, 48 h; for **5d**, Me_2NH (5.5 equiv), EtOH, rt, 24 h, yield 65%. (c) For **6−12**, R^2X, CH_3CN or acetone; for **13**, FCH_2CH_2OTf, CH_2Cl_2 followed by Amberlite IRA-410 (Cl). (d) Amberlite IRA-410 (OH). (e) Aqueous HCl, dioxane, 40−70 °C, yield 70% (on **5d**).

a column with ion-exchange resin Amberlite IRA-410 (OH form) to give the target phosphinates **78** and **79**.

The tetrazole analogue **82** of GBB was synthesized by $AlCl_3$-catalyzed [2 + 3] cycloaddition reaction of 4-chlorobutyronitrile (**80**) and sodium azide, followed by condensation of the obtained intermediate 5-(3-chloropropyl)tetrazole (**81**) with trimethylamine (Scheme 10).

The higher homologue of GBB **85**,[19] bearing one more methylene unit in the carbon chain, was prepared from 5-bromopentanoic acid (**83**) (Scheme 11) by treatment with trimethylamine and further conversion of the obtained bromide **84** into betaine **85** by use of ion-exchange resin Amberlite IRA-410 (OH form).

Preparation of 1-substituted (1,1-dimethylhydrazin-1-ium-2-yl)propanoate analogues **91−97** (Scheme 12 and Table 1) started from methyl 3-(2,2-dimethylhydrazinyl)propanoate (**87**),[20] which was alkylated exclusively at the tertiary nitrogen atom by appropriate alkyl bromides. The corresponding ester hydrazinium salts **88a−f**, were converted into betaines **91−95** with the strongly basic anion-exchange resin Amberlite IRA-410 (OH form), or, alternatively, by acidic hydrolysis with ca. 2.5 N HCl at 70−75 °C into hydrazinium salts **96** and **97**. As in the synthesis of **52** (Scheme 3), 2-chloroethyl derivative **88e** upon ion-exchange resin treatment formed vinyl derivative **95**, and we were able to prepare the corresponding acid **96** with 2-chloroethyl substituent intact only under acidic hydrolysis conditions. Methyl 3-(1,2,2-trimethylhydrazinyl)propanoate **89a** and its *N*-ethyl analogue **89b**, necessary for further synthesis of tetrasubstituted hydrazinium derivatives **98−101**, were obtained by Michael addition of trimethylhydrazine to methyl acrylate[21] or by reductive amination protocol of dimethylhydrazinyl propanoate **87** with acetaldehyde/sodium cyanoborohydride, respectively. The obtained tetrasubstituted hydrazines **89a** and **89b** were alkylated with appropriate alkyl iodides mainly at *N*-(2) position to afford the expected hydrazinium salts **90a−90d**, which were hydrolyzed with anion-exchange resin Amberlite IRA-410 (OH form) into target betaines **98−101** (Scheme 12).

N,*N*-Diethyl analogue **104** of mildronate was prepared from known[22] methyl 3-(2,2-diethylhydrazinyl)propanoate (**102**) by alkylation of the latter with iodomethane, followed by hydrolysis of the obtained hydrazinium ester **103** by Amberlite IRA-410 (OH form) treatment (Scheme 13). Since we could not isolate the prepared betaine in a crystalline form, the product was converted into chloride salt **104**.

To obtain the 2-aminoethyl analogue of mildronate **106** (Scheme 14), methyl 3-(2,2-dimethylhydrazinyl)propanoate

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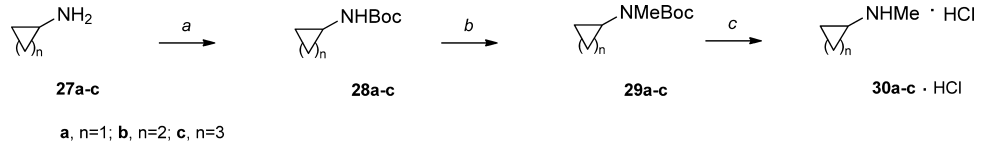
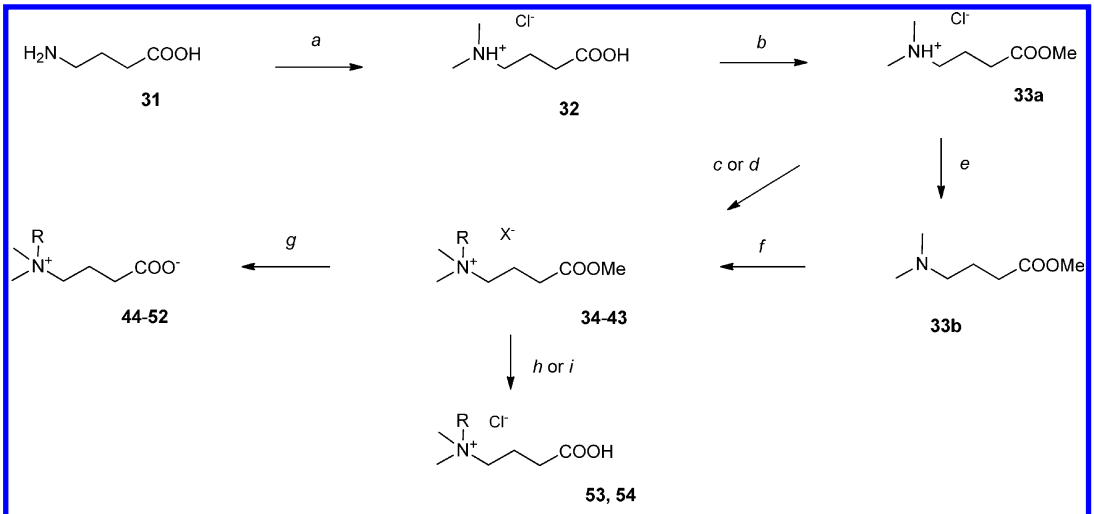
Scheme 2. Synthesis of Secondary Alicyclic Methylamines 30a−c[a]



[a]Reagents and conditions: (a) Boc_2O, CH_2Cl_2, 0 °C to rt; yields 100% (**28a**), 91% (**28b**), and 77% (**28c**). (b) NaH, MeI, DMF, rt, overnight; yields 53% (**29a**), 46% (**29b**), and 36% (**29c**). (c) HCl, MeOH, rt, 4 h; yields 96% (**30a**), 90% (**30b**), and 100% (**29c**).

Scheme 3. Synthesis of GBB Analogues 44−54 from 4-Aminobutanoic Acid (31)[a]



No	X [b]	R
34, 44	Br	CH_3
35, 45	Br	CH_2
36, 46	Br	CH_3, CH_3
37, 47	Br	CH
38, 48	Cl	Cl
39, 49	Br	Br

[b] For compounds **34-39**

No	X [b]	R
40, 50	I	I
41, 51	Cl	OMe
52		CH_2
42, 53	Br	Cl
43, 54	Br	Br

[b] For compounds **40-43**

[a]Reagents and conditions: (a) CH_2O, HCO_2H, reflux, yield 69−83%. (b) $SOCl_2$, MeOH, −10 °C to rt, then 40−50 °C, yield 77%. (c) For **34**, *n*-PrBr, K_2CO_3, acetone, rt, 5 days, yield 68%. (d) For **35**, $BrCH_2CH{=}CH_2$, $NaHCO_3$, acetone, rt, 13 days, yield 88%. (e) K_2CO_3, CH_2Cl_2, rt, 24 h, then distillation at 32−35 °C/3−4 mmHg, yield 66%. (f) For **36−43**, RX, neat, CH_3CN, or acetone. (g) Amberlite IRA-410 (OH). (h) For **53**, Amberlite IRA-410 (Cl), followed by 1 N HCl, 70 °C, yield 79%. (i) For **54**, 2 N HCl, 85 °C, followed by Amberlite IRA-410 (Cl), yield 79%.

(**87**) was treated with tert-butyl (2-bromoethyl)carbamate and the produced hydrazinium ester was hydrolyzed with Amberlite IRA-410 (OH form), yielding Boc-protected betaine **105**. The Boc-protecting group was removed by 6 N HCl and the obtained product was converted to the target betaine **106** by use of anion-exchange resin as above.

Synthesis of a sulfonate analogue of mildronate **110** (Scheme 15) consisted of reaction of 1,1-dimethylhydrazine with easily available[23] isopropyl ethenesulfonate (**107**), yielding the expected dimethylhydrazinyl conjugate addition product **108**. Compound **108** was alkylated at the tertiary nitrogen atom with iodomethane to form the trimethylhydrazinium derivative **109**, which, similarly to the carboxylates above, was converted into target betaine **110** with Amberlite IRA-410 (OH form).

The phosphonic acid analogue of mildronate **114** was obtained from known[24] diethyl [2-(2,2-dimethylhydrazono)ethyl]phosphonate

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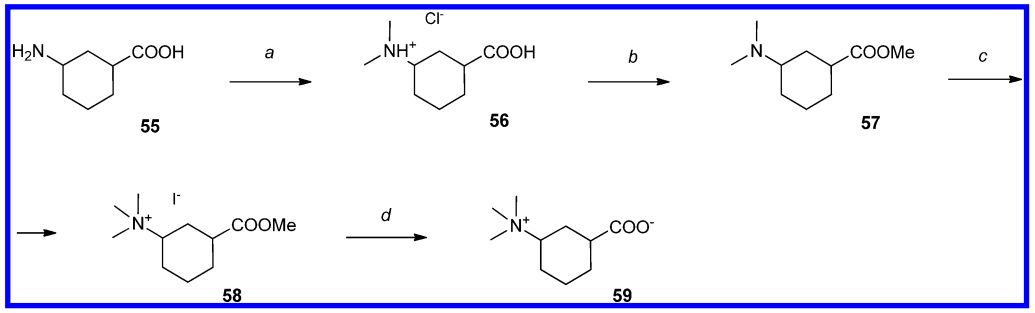
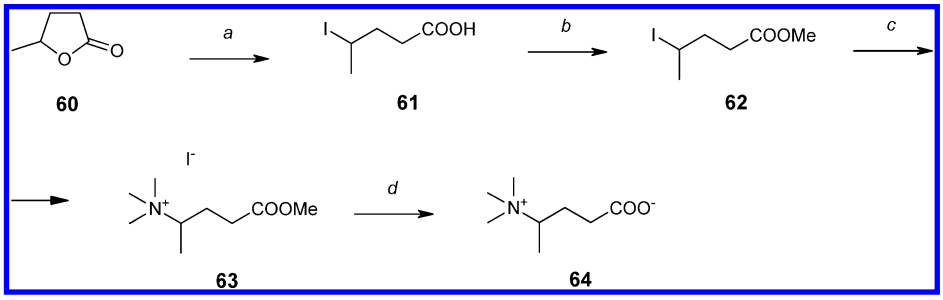
Scheme 4. Synthesis of 3-(Trimethylammonio)cyclohexanecarboxylate (59)[a]



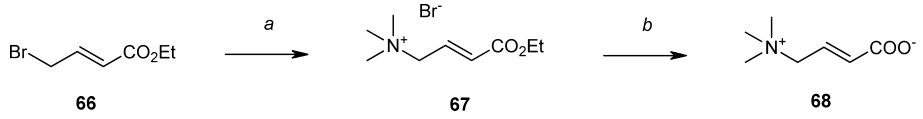
[a]Reagents and conditions: (a) CH_2O, HCO_2H, reflux, followed by HCl, yield 93%. (b) AcCl, MeOH, 42 °C, followed by K_2CO_3, CH_2Cl_2, rt, yield 76%. (c) MeI, MeOH, rt, yield 91%. (d) Amberlite IRA-410 (OH), yield 91%.

Scheme 5. Synthesis of 4-(Trimethylammonio)pentanoate (64)[a]



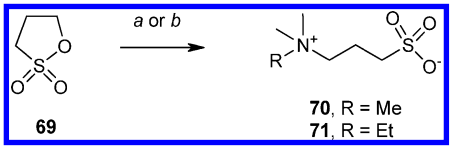
[a]Reagents and conditions: (a) Me_3SiCl, NaI, CH_3CN, 80 °C, yield 66%. (b) AcCl, MeOH, rt, yield 70%. (c) Me_3N, EtOH, 50 °C, 48 h. (d) Amberlite IRA-410 (OH), yield 32% (on **62**).

Scheme 6. Synthesis of (*E*)-4-(Trimethylammonio)but-2-enoate (68)[a]



[a]Reagents and conditions: (a) Me_3N, EtOH, rt, 5 days, yield 83%. (b) Amberlite IRA-410 (OH), yield 72%.

Scheme 7. Aminolysis of 1,3-Propanesultone (69) by Trimethylamine and Dimethylethylamine[a]



[a]Reagents and conditions: (a) For **70**, Me_3N, benzene, rt, 3 days, yield 31%. (b) For **71**, Me_2NEt, 1,2-dichloroethane, rt, 2 days, yield 59%.

(**111**) by reduction of the latter with sodium cyanoborohydride, followed by methylation of the obtained dimethylhydrazinyl phosphonate **112** with iodomethane to afford the expected trimethylhydrazinium salt **113** and acidic hydrolysis of the diethyl phosphonate moiety of the salt **113** with 6 N HCl at reflux temperature (Scheme 16).

Structure–Activity Relationship Considerations and Crystal Structures of Selected Inhibitors in Complex with γ-Butyrobetaine Hydroxylase. *Modifications of Trimethylammonium Group.* Compound **14** shares the structure of GBB, with an ethyl group replacing a methyl group in the trimethylammonium moiety; it has an approximately 20-fold lower IC_{50} than mildronate. The binding of **14** to BBOX was studied by X-ray crystallography. Electron density maps revealed that **14** binds in the same pose as GBB. Furthermore, even at 1.7 Å resolution, no obvious density for the additional carbon was apparent (data not shown, due to essentially identical electron density for the ligand compared with the GBB/BBOX complex; PDB code 3O2G). Presumably, the ethyl group may occupy any of three positions, and the increased affinity is due to a slightly larger contact area. Despite the apparently random location of the ethyl group, replacing two methyl groups with ethyl groups, which was done in **15**, resulted in significant reduction of potency (IC_{50} = 100 μM). However, restricting flexibility and removing hydrogen atoms from the two ethyl groups by joining them in a pyrrolidine ring (e.g., pyrrolidinium derivative **26**) yielded an even more potent compound (IC_{50} = 0.49 μM) than **14**. The crystal structure of **26** in complex with BBOX revealed the pyrrolidinium moiety in a well-defined orientation, partially filling the cavity A (Figure 2C). The backbone of the inhibitor adopted a different conformation, occupying more volume in cavity B as well.

As mentioned above, the positively charged ammonium group is crucial for binding. Thus, unsurprisingly, 1-(3-carboxypropyl)-pyrrolidin-1-ium chloride (**25**), which at physiological pH should be neutral, does not inhibit BBOX, unlike its *N*-methylpyrrolidinium congener **26.**

With replacement of a single methyl group in GBB analogues, some increase in the steric bulk of the ethyl group is allowed. Thus, replacement of the ethyl group in **14** by cyclopropyl or

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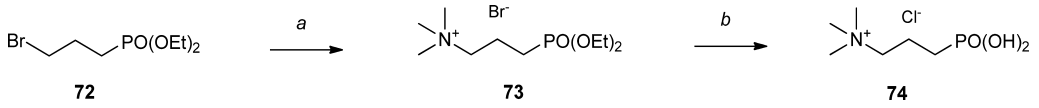
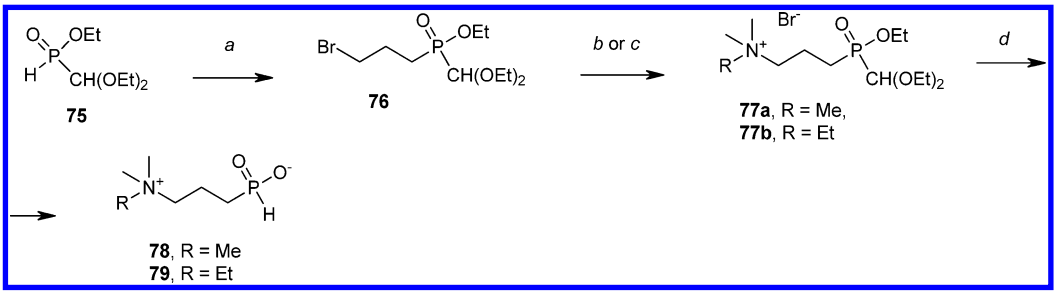
Scheme 8. Synthesis of Phosphonic Acid Analogue 74 of GBB[a]



72 73 74

[a]Reagents and conditions: (a) Me_3N, EtOH, rt, 5 days, yield 96%. (b) Amberlyst 21 (Cl), followed by concd HCl, reflux, 24 h, yield 70%.

Scheme 9. Synthesis of Phosphinic Acid Analogues 78 and 79 of GBB[a]

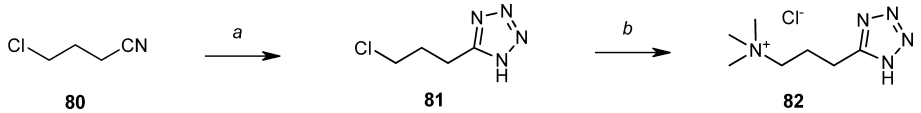


75 76 77a, R = Me, 77b, R = Et

78, R = Me
79, R = Et

[a]Reagents and conditions: (a) NaH, THF, then $Br(CH_2)_3Br$, 0 °C to rt, yield 23%. (b) For **77a**, Me_3N, EtOH, CH_3CN, rt, 4 days. (c) For **77b**, Me_2NEt, CH_3CN, rt, 9 days. (d) (Concd HCl, reflux, 22 h, followed by Amberlite IRA-410 (OH), yield 62% (**78**, calculated with respect to **76**) and 47% (**79**, calculated with respect to **76**).

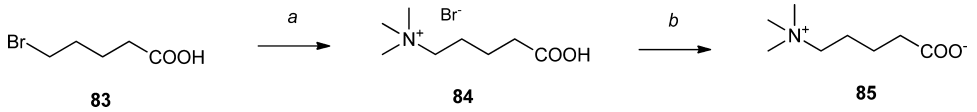
Scheme 10. Synthesis of Tetrazole Analogue 82 of GBB[a]



80 81 82

[a]Reagents and conditions: (a) NaN_3, $AlCl_3$, THF, reflux, 30 h, yield 66%. (b) Me_3N, EtOH, 55 °C, 78 h, yield 51%.

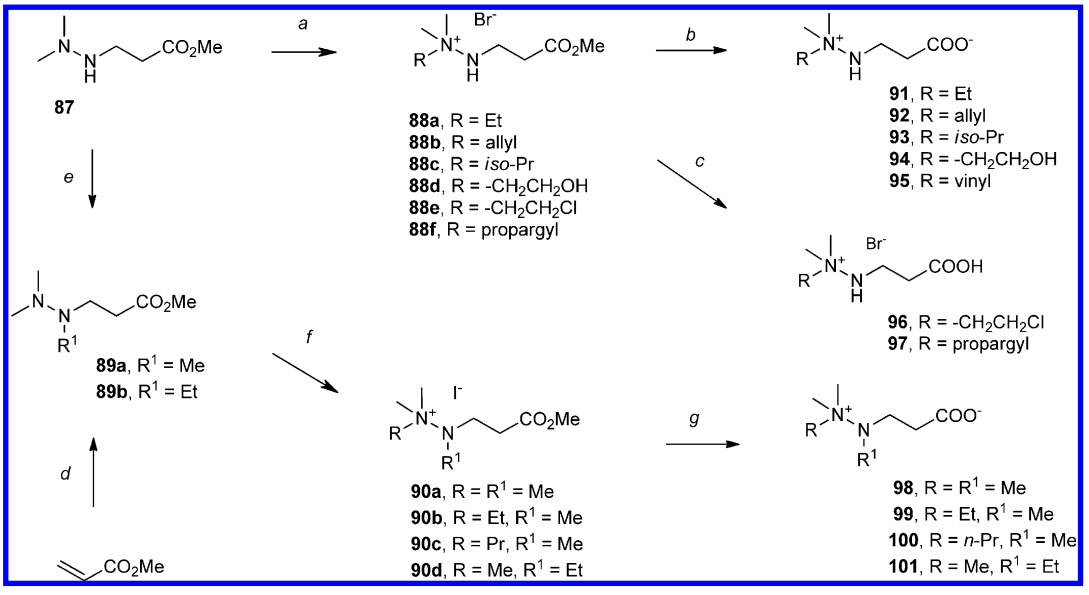
Scheme 11. Synthesis of 5-(Trimethylammonio)pentanoate (85)[a]



83 84 85

[a]Reagents and conditions: (a) Me_3N, EtOH, CH_3CN, rt, 2 days, yield 75%. (b) Amberlite IRA-410 (OH), yield 61%.

Scheme 12. Synthesis of Mildronate Analogues 91−101[a]



[a]Reagents and conditions: (a) RBr, EtOH, rt. (b) Amberlite IRA-410 (OH). (c) ca. 2.4 N HCl, 75 °C, 2 h, yield 68% (**96**) or 71% (**97**). (d) Me_2NNHMe, reflux, 24 h, yield 69%. (e) CH_3CHO, $NaBH_3CN$, MeOH, rt, yield 68%. (f) RI, EtOH, rt. (g) Amberlite IRA-410 (OH).

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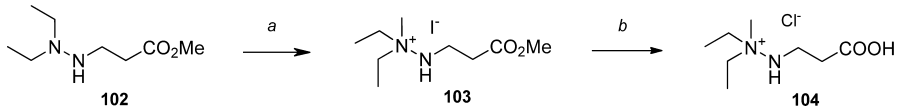
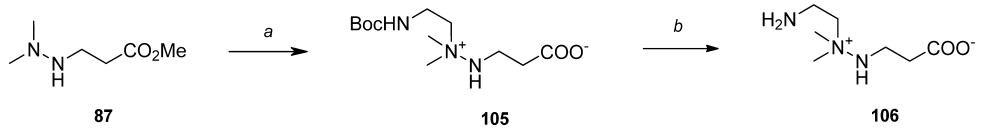
Scheme 13. Synthesis of 2-(2-Carboxyethyl)-1,1-diethyl-1-methylhydrazin-1-ium Chloride (104)[a]



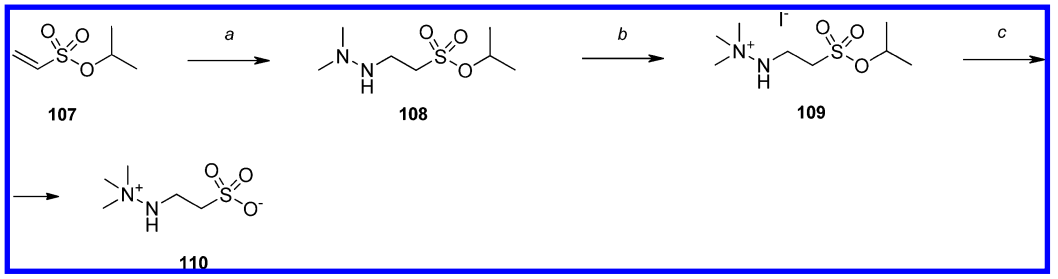
[a]Reagents and conditions: (a) MeI, EtOH, rt, yield 72%. (b) Amberlite IRA-410 (OH), followed by HCl, rt, yield 74%.

Scheme 14. Synthesis of 3-[1-(2-Aminoethyl)-1,1-dimethylhydrazin-1-ium-2-yl]propanoate (106)[a]



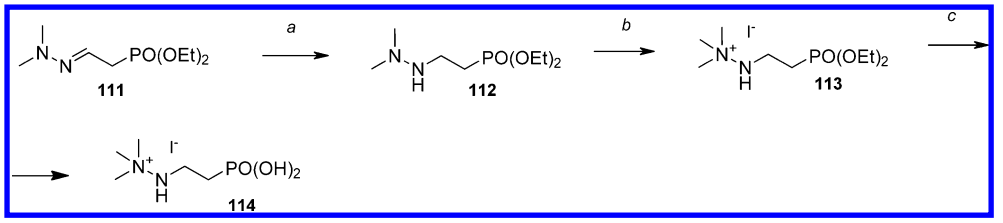
[a]Reagents and conditions: (a) $Br(CH_2)_2NHBoc$ (neat), rt, followed by Amberlite IRA-410 (OH), yield 69%. (b) 6 N HCl, rt, followed by Amberlite IRA-410 (OH), yield 67%.

Scheme 15. Synthesis of Sulfonate Analogue of Mildronate (110)[a]



[a]Reagents and conditions: (a) Me_2NNH_2 (neat. (b) MeI, EtOH, rt, yield 72% (on **107**). (c) Amberlite IRA-410 (OH), yield 80%.

Scheme 16. Synthesis of Phosphonic Acid Analogue 114 of Mildronate[a]



[a]Reagents and conditions: (a) $NaBH_3CN$, MeOH, followed by 1 N HCl, MeOH to pH 3−4, rt, overnight, yield 82% (crude). (b) MeI, EtOH, rt, yield 67%. (c) 6 N HCl, reflux, 15 h, yield 72%.

cyclobutyl groups produced **19** and **20**, with improved potencies (IC_{50} = 2.1 and 0.81 μM, respectively). However, an isopropyl substituent as in **46** already decreased potency (IC_{50} = 5.7 μM). A further increase in steric bulk was not tolerated: introduction of propyl (**44**), cyclopentyl (**21**), cyclopropylmethyl (**22**), cyclobutylmethyl (**23**), and butyl (**17**) groups resulted in completely lost potency, which is not surprising given the small volume of cavity A.

Replacement of the 1-methyl group of the 1,1,1-trimethylhydrazin-1-ium-2-yl portion of mildronate with other saturated aliphatic groups yielded less potent inhibitors. Replacement of one methyl group of mildronate with an ethyl group resulted in reduction of potency (**91**, IC_{50} = 140 μM); isopropyl substitution yielded the inactive **93** (IC_{50} >1000 μM). Replacement of two methyl groups in mildronate with ethyl groups resulted in an inactive compound, **104** (IC_{50} = >1000 μM).

GBB analogues containing unsaturated bonds (which could increase affinity via possible $\pi-\pi$ interactions) reduced BBOX activity in the following order: propargyl **47** (IC_{50} = 1.4 μM) > allyl **45** (IC_{50} = 2.7 μM) > vinyl **52** (IC_{50} = 127 μM) ≫ benzyl **18** (inactive). In contrast, among similar mildronate analogues, vinyl derivative **95** exhibited 9-fold higher potency (IC_{50} = 6.8 μM) than the parent compound. Propargyl compound **97** was more potent (IC_{50} = 24 μM) than mildronate, but allyl substitution produced a less active analogue **92** (IC_{50} = 119 μM).

The introduction of a 2-halogen atom in the ethyl group of GBB analogue **14** resulted in compounds with increased potency. Thus, **24** (with a 2-fluoroethyl substituent) exhibited IC_{50} = 610 nM, which is approximately 5 times better than for **14**. The crystal structure of **24** in complex with BBOX revealed that the 2-fluoroethyl group occupies cavity A and that the fluorine atom forms a hydrogen bond with the hydroxyl group of Tyr366 (Figure 2B). Replacement of the fluorine atom at the 2-position with chlorine resulted in further increase in potency (**53**, IC_{50} = 260 nM), but the inhibitory potency was reduced by the bulkier bromine atom (**54**, IC_{50} = 1.2 μM).

The only available compound with a similar structure among mildronate analogues is 2-chloroethyl compound **96**, which exhibits weaker inhibitory potency (IC_{50} = 10.2 μM) than the corresponding GBB analogue but remarkably higher than the parent mildronate. Replacement of hydrogen atoms in the GBB methyl groups with halogens did not increase BBOX inhibition;

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the corresponding halomethyl analogues possessed only weak inhibitory potencies, which decreased with increasing size of the halogen atom: 2-chloromethyl **48** (IC_{50} = 268 μM) > 2-bromomethyl **49** and 2-iodomethyl **50** (inactive).

Incorporation of hydrogen-bond donor/acceptor groups at the 2-position of the ethyl group did not result in active compounds; thus, 2-hydroxyethyl analogues of GBB and mildronate (**16** and **94**, respectively) and the 2-aminoethyl mildronate derivative **106** did not possess any inhibitory potency.

In changing from a trimethylammonium group to a dimethylsulfonium group, despite the loss of one methyl group, ~15% increase in total volume[25] is expected, which may better fill cavity A. Although a dimethylsulfonium analogue of GBB (**86**) was prepared, it demonstrated only weak BBOX inhibitory potency (IC_{50} = 388 μM). Presumably, the dimethylsulfonium moiety can participate in similar cation−π interactions as the trimethylammonium groups of GBB and mildronate, but the increase in atomic radius, together with the distinctive symmetry of this group, are not beneficial.

Modifications of Core Region. Earlier attempts to modify the core of GBB with cyclopropyl groups[26] are consistent with crystallographic evidence of an unexploited "cavity B". We attempted to exploit cavity B with a number of new compounds. Crystal structures were determined for two of them, **98** and **101**. Compound **98**, our best BBOX inhibitor (IC_{50} = 90 nM), has a methyl group at N-2, which partially fills cavity B (Figure 2E). In the hydrazinium series, this position coincides with the C-4 position of the GBB analogues. Compound **101** is similar but with an ethyl group attached at N-2. The electron density for **101** is not as well-defined as that for **98**, indicating that **101** binds in two conformations, with the ethyl group precluding optimal contact in both cases (data not shown due to poor electron density). These results are also reflected in the IC_{50} values, with **101** being less active (IC_{50} = 2.8 μM). Efforts to further modify our most potent inhibitor, **98**, have so far resulted only in less active compounds. Thus, replacement of a 1-methyl group of the 1,1,1,2-tetramethylhydrazin-1-ium-2-yl moiety with an ethyl group resulted in a 80-fold less potent compound, **99** (IC_{50} = 7.2 μM); substitution with an *n*-propyl group also afforded a less potent compound, **100** (IC_{50} = 3.1 μM). It is interesting to note that a similar reduction in potency with 1-ethyl substitution was also observed for 2-N-unsubstituted analogue **91**. Contrary to the mildronate series, the introduction of an extra methyl group at the C-4 position of GBB resulted in **64**, with low potency (IC_{50} = 343 μM).

Modifications of the GBB core, such as introduction of an extra chloro substituent at C-3 or replacement of 4-butanoate with 3-cyclohexanecarboxylate, gave weakly active **65** (IC_{50} = 459 μM) and inactive **59**, respectively. However, replacement of the butanoate moiety of GBB with 4-crotonate resulted in the active unsaturated analogue **68** (IC_{50} = 3.8 μM).

Modifications of Carboxylic Acid. Carboxyl groups on mildronate and GBB form hydrogen bonds with Asn 191, Asn 292, and the backbone nitrogen atom of Tyr205. Theoretically, it seems plausible to replace the carboxylate with other hydrogen-bond donors or acceptors; however, it appears that an acidic group is required in this part of a substrate or inhibitor to maintain potency. For example, the amide of **14** is inactive (data not shown). An attempt to replace the carboxylate of GBB with a tetrazole group also produced an inactive compound, **82**. However, changing the carboxylate to some other bioisosteric groups produced active compounds: for example, phosphonate **74** (IC_{50} = 100 nM, our second best BBOX inhibitor so far) and, to a lesser extent, sulfonate **70** (IC_{50} = 1.7 μM). The second most potent inhibitor in this group was phosphinate **78** (IC_{50} = 520 nM). The crystal structure of BBOX in complex with **78** revealed that the phosphinate moiety was positioned in approximately the same position as the carboxylate in GBB and mildronate (Figure 2F). However, the backbone assumed a different conformation, partially occupying cavity B. We believe this is why **78** has such high potency. The replacement of one methyl group in the trimethylammonium moiety of GBB sulfonate **70** with an ethyl group resulted in decreased potency of **71** (IC_{50} = 6.7 μM), but the same replacement in phosphinate **78** had no effect, since **78** and **79** (IC_{50} = 530 nM) displayed almost identical potencies. In contrast to the GBB series, where phosphonates were more active than sulfonates, in the mildronate series this was reversed: sulfonate **110** (IC_{50} = 87 μM) was slightly more active than phosphonate **114** (IC_{50} = 117 μM).

γ-Butyrobetaine and Mildronate Analogues. Mildronate is a GBB analogue with nitrogen in place of C-4. We synthesized a number of compounds with C-4 intact (GBB analogues) or with nitrogen at this position (mildronate analogues). For several otherwise identical GBB/mildronate analogues, the IC_{50} values were very different. For example, **14** (a GBB analogue) was highly active (IC_{50} = 3.3 μM), but the corresponding mildronate analogue, **91**, was significantly less active (IC_{50} = 140 μM). Interestingly, the situation was reversed for some compounds: **98** (a mildronate analogue) had very high inhibitory potency (IC_{50} = 90 nM), whereas **64** (a GBB analogue) exhibited a spectacular reduction in potency (IC_{50} = 343 μM). The crystal structures of **98** and **64** revealed that both adopted almost identical poses in the active site (Figure 3). Thus, large IC_{50} differences between mildronate and GBB analogues cannot be explained solely by structural characteristics.

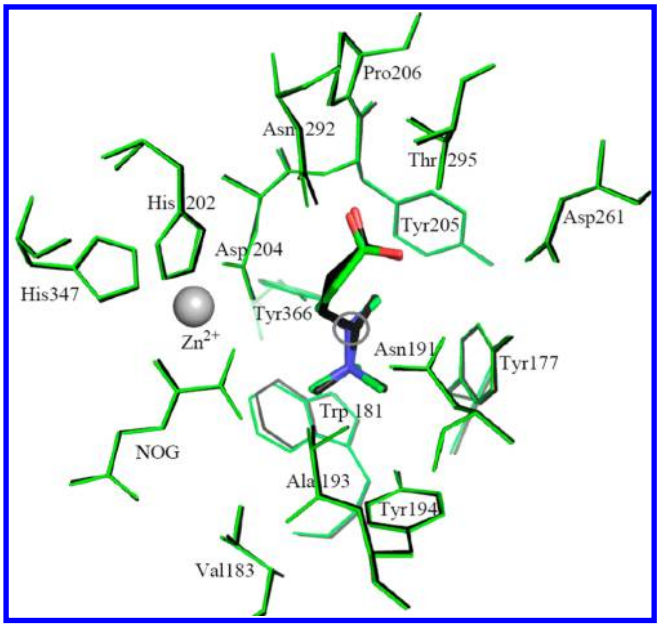

Figure 3. Nearly identical binding modes of compounds with different inhibitory constants. Compounds **98** (stick model with green carbon atoms) and **64** (black carbon atoms) differ only by having nitrogen (**98**) or carbon (**64**) atoms in position 4 (denoted with circle). Both compounds are bound in the active site in a near-identical conformation; moreover, conformations of nearby protein residues (thinner stick models) are essentially identical. The IC_{50} values of **98** and **64** differ by almost 3 orders of magnitude, while K_d values are similar.

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We used isothermal calorimetry (ITC) to ascertain whether large differences in IC_{50} for GBB and mildronate analogues resulted from differences in dissociation constants. In order to prevent any enzymatic reaction, in our ITC experiments native cofactor and ion were replaced with isosteric ones: 2-ketoglutarate with *N*-oxalylglycine and Fe(II) with Ni(II). This could potentially influence inhibitor binding, but we consider that major changes in K_d values due to cofactor surrogates are unlikely.We focused on inhibitors **98** and **64**, the most striking example of structurally similar GBB/mildronate analogues with different IC_{50} values. However, the K_d values for **64** (4.1 μM) and **98** (9.3 μM) were surprisingly similar. Thus, binding and X-ray studies indicate that these compounds bind similarly; differences in IC_{50} may be related to subsequent events.

Initially, it was believed that mildronate inhibited BBOX rather than acting as a substrate. However, Leung et al.[12] reported that mildronate is a BBOX substrate. Several products were identified, such as malonic acid semialdehyde, dimethylamine, 3-amino-4-(methylamino)butanoic acid, and formaldehyde. In contrast, GBB is exclusively converted to carnitine, with no other products observed. Similarly, mildronate analogues may also form a number of products. This factor may explain the large differences observed in IC_{50} values; the reaction products may also act as inhibitors or inactivators. Alternatively, some inhibitor-dependent allosteric regulation may take place. No evidence of additional binding sites could be observed in the structures of BBOX in complex with any inhibitor, even at the high inhibitor concentrations necessary for crystallization. Because BBOX is a dimer, an inhibitor might bind to one monomer, subsequently inducing subtle changes in the other monomer. We consider this unlikely because the active sites are far each from other, and the conformation of the protein was essentially identical in complex with all inhibitors. We were also unable to detect any asymmetry between the binding pockets because there is only one monomer in the asymmetric unit. If asymmetry in the binding sites does not perturb crystal symmetry, it should result in smeared density or alternative conformations in the active-site residues because the final structure reflects the average of both monomers. However, asymmetric binding is unlikely because the electron density is well-defined for all active-site residues, and no alternative conformations are apparent near the active site.

Selected compounds from this study have been tested for anti-ischemic activity in rat hearts in vivo and ex vivo and patented for the treatment of cardiovascular diseases.[27,28] Thus the inhibition of BBOX represents an effective strategy to protect the heart against ischemia−reperfusion-induced damage.

CONCLUSION

We have designed, synthesized, and characterized 51 compounds, among which 36 are novel; 23 of these display superior BBOX inhibition relative to the commercially available mildronate. Crystal structures of several inhibitors, which were solved in complex with BBOX, revealed that these compounds fill the active-site cavity better than mildronate. We also observed that some structurally similar GBB/mildronate analogues are bound to BBOX in essentially an identical fashion and display similar dissociation constants but still have very different IC_{50} values.

EXPERIMENTAL SECTION

Protein Production and Purification. The BBOX gene, in addition to the encoding sequence for the 6×His tag and TEV protease cleavage site, was PCR-amplified and cloned in yeast expression vector pFX7[29] under control of the hybrid GAL10-PYK1 galactose-inducible promoter. The resulting construct was transformed in *Saccharomyces cerevisiae* strain AH22 (MATa leu2 his4) and expressed as previously described.[30] The cells were collected by low-speed centrifugation, washed once with distilled H_2O, and stored at −20 °C until used.

To purify the target protein, cells were resuspended in lysis buffer, which contained 20 mM Tris-HCl and 300 mM NaCl at pH = 8.0 (4 mL of buffer/1 g of wet cells), and disrupted with a French press (three cycles, 20 000 psi). After centrifugation for 30 min at 18500*g*, the supernatant was manually passed through the HisTrap FF crude column, which was pre-equilibrated in lysis buffer. The column was then washed with lysis buffer that contained 20 mM imidazole, and the bound proteins including BBOX were eluted with lysis buffer containing 300 mM imidazole.

To remove excess salt and imidazole, the eluent was passed through a HiPrep 26/10 desalting column in 20 mM Tris-HCl (pH = 8.0), which was connected to an ÄKTA chromatography device (Amersham Biosciences). Finally, the protein mixture was loaded onto a Mono Q 5/50 GL column, and the bound proteins were separated by elution with a linear salt gradient (0−1 M NaCl in 20 column volumes). BBOX peak appeared at about 300 mM NaCl concentration. To remove the 6×His tag, the recombinant TEV protease (100 μg of protease/2 mg of protein) in 0.5 mM ethylenediaminetetraacetic acid (EDTA) and 1 mM dithiothreitol (DTT) (final concentration) was added and cleavage was performed for 72 h at 4 °C. Salts were removed via HiPrep 26/10 desalting column pre-equilibrated in 20 mM Tris-HCl (pH = 8.0). Finally, the mixture was passed through a HisTrap FF crude column. The flowthrough contained only cleaved BBOX since the 6×His tag, remains of the uncleaved protein along with the TEV protease, remained bound to the column due to presence of histidine repeats. All the prepacked columns used were purchased from GE Healthcare.

Chemistry. NMR spectra were recorded at ambient temperature on a Varian 400 Oxford NMR spectrometer (400 MHz). Liquid chromatography−mass spectrometry (LC−MS) was performed on an Acquity ultraperformance liquid chromatography (UPLC) system (Waters) connected to a Micromass Quatro micro API tandem mass spectrometer (Micromass) operating in the ESI (electrospray ionization) positive-ion mode and an Acquity UPLC BEH HILIC column (1.7 μm, 2.1 × 100 mm) with a gradient of 80−50% acetonitrile/10 mM ammonium acetate (pH 4). High-resolution mass spectrometry (HRMS) was carried out on a Micromass Q-Tof micro mass spectrometer. Gas chromatography (GC)−MS analyses were performed on an Agilent Technologies 7890A gas chromatographic system with Agilent Technologies 5975C mass-selective detector (MSD). IR spectra were recorded on a Shimadzu Fourier transform infrared (FTIR) Prestige-21 spectrometer. Elemental analyses were obtained with a Carlo Erba EA 1108 instrument. Melting points were measured on Gallenkamp or OptiMelt melting point apparatus and are uncorrected. Reactions leading to polar intermediates and target structures as betaines and salts (covered by Table 1) were monitored by thin-layer chromatography (TLC) on DC-Alufolien Kieselgel 60 F254 plates (Merck) using the following solvent systems: (a) chloroform/methanol (9:1), (b) acetonitrile/water/acetic acid (60:40:1), and (c) methanol/20% aqueous NH_4OH (7:3). The spots were visualized with ninhydrin (0.5 g) and acetic acid (1 mL) solution in ethanol (100 mL) with subsequent heating at 120 °C. For halogen exchange experiments and synthesis of betaines by alkaline hydrolysis of the corresponding salts, not less than 4-fold amount (theoretical) of ion-exchange resin (total exchange capacity ≥1.25 equiv/L as stated by producer) was used.

Generally, target compounds were obtained by evaporating water solutions thereof. The obtained oil was azeotropically dried with 2-propanol, acetonitrile, and/or acetone until solidified and then triturated in the ultrasound bath with ether and/or acetone. The solid material was dried in vacuo (ca. 1 mmHg) over P_2O_5 to constant weight prior to C, H, N analysis. Samples for biological experiments were immediately closed and exact weight of the compound was registered. The content of the vial was further dissolved in water or appropriate buffer solution for biological experiments. The amount of water was calculated from elemental analysis data and taken into account in the preparation of solutions for biological tests.

The purity of synthesized compounds was checked by LC/MS. All compounds have shown purity >95% (area percent, ESI+, TIC mode). Content of halide counterions in synthesized salts **24**–**26**, **53**, **54**, **65**, **74**, **82**, **96**, **97**, and **104** was determined by argentometric titration with 0.1 N silver nitrate. Titrant was standardized with sodium chloride solution before use.

All reagents and solvents were purchased from Sigma–Aldrich, Acros Organic, Alfa Aesar, TCI Europe, and Apollo Scientific. Silica (0.035–0.070 mm, Acros) was used for column chromatography. Reaction conditions and yields were not optimized. All solvents were purified before use by routine techniques.

4-Ethoxy-*N*-ethyl-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (2). To a solution of ethyl 4-bromobutanoate (**1**) (19.5 g, 0.1 mol) in dichloromethane at ice-bath temperature was added *N*,*N*-dimethylethylamine (10.8 mL, 0.1 mol), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated, and the residue was triturated with acetone (50 mL) and kept at 0 °C for 0.5 h. The precipitate was filtered and dried in vacuo over P_2O_5 to afford **2** (22.274 g, 94%) as white hygroscopic crystals, mp 80.8–99.0 °C. ^{1}H NMR ($CDCl_3$, hexamethyldisiloxane (HMDSO)) δ 1.26 (t, J = 7.2 Hz, 3H), 1.44 (t, J = 7.4 Hz, 3H), 2.00–2.11 (m, 2H), 2.52 (t, J = 6.6 Hz, 2H), 3.40 (s, 6H), 3.64–3.73 (m, 2H), 3.69 (q, J = 7.4 Hz, 2H), 4.14 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O) δ 7.4, 13.3, 17.4, 30.2, 50.0 [t, $^1J_{(N,C)}$ = 4.0 Hz], 59.6 [t, $^1J_{(N,C)}$ = 2.6 Hz], 61.9, 62.1 [t, $^1J_{(N,C)}$ = 3.0 Hz], 174.6. LC–MS (ESI) m/z 188 $[M - Br^-]^+$. Anal. Calcd for $C_{10}H_{22}BrNO_2 \cdot 0.67H_2O$: C 42.86, H 8.39, N 5.00. Found: C 42.86, H 8.54, N 4.97.

4-Ethoxy-*N*,*N*-diethyl-*N*-methyl-4-oxobutan-1-aminium Bromide (3). A mixture of ethyl 4-bromobutanoate (**1**) (9.670, 49.57 mmol) and diethylmethylamine (10.8 g, 123.9 mmol) in acetone (15 mL) was stirred at room temperature for 8 days. The reaction mixture was supplemented with diethyl ether (70 mL), and the precipitate was filtered, washed with diethyl ether, and dried in vacuo over P_2O_5 to give **3** (12.16 g, 87%) as white crystals, mp 126–129 °C. ^{1}H NMR (D_2O, 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS)) δ 1.27 (t, J = 7.2 Hz, 3H), 1.33 (t, J = 7.1 Hz, 6H), 2.04 (m, 2H), 2.52 (t, J = 6.9 Hz, 2H), 2.99 (s, 3H), 3.27 (m, 2H), 3.37 (q, J = 7.1 Hz, 4H), 4.19 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O) δ 7.0, 13.2, 16.9, 30.2, 46.8 [t, $^1J_{(N,C)}$ = 3.9 Hz], 56.5 [t, $^1J_{(N,C)}$ = 2.6 Hz], 58.9 [t, $^1J_{(N,C)}$ = 3.0 Hz], 61.9, 174.6. LC–MS (ESI) m/z 202 $[M - Br^-]^+$. Anal. Calcd for $C_{11}H_{24}BrNO_2$: C 46.81, H 8.57, N 4.96. Found: C 46.70, H 8.72, N 4.97.

4-Ethoxy-*N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (4). Compound **4** was obtained from ethyl 4-bromobutanoate (**1**) and 2-(dimethylamino)ethanol by a similar protocol as **2**; yield 52%, mp 100–102 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.26 (t, J = 7.1 Hz, 3H), 2.10 (m, 2H), 2.49 (t, J = 6.8 Hz, 2H), 3.39 (s, 6H), 3.69 (m, 2H), 3.77 (m, 2H), 4.14 (q, J = 7.1 Hz, 2H), 4.15 (m, 2H), 5.04 (br s, 1H). ^{13}C NMR (D_2O) δ 13.2, 17.6, 30.2, 51.4 [t, $^1J_{(N,C)}$ = 3.8 Hz], 55.3, 61.9, 64.0 [t, $^1J_{(N,C)}$ = 2.8 Hz], 64.9 [t, $^1J_{(N,C)}$ = 2.8 Hz], 174.5. LC–MS (ESI) m/z 204 $[M - Br^-]^+$. Anal. Calcd for $C_{10}H_{22}BrNO_3$: C 42.26, H 7.80, N 4.93. Found: C 42.07, H 7.82, N 4.84.

Ethyl 4-[Cyclopropyl(methyl)amino]butanoate (5a). To a solution of *N*-methylcyclopropanamine hydrochloride (**30a**) (2.299 g, 21.37 mmol) in acetonitrile (50 mL) were added successively triethylamine (4.0 mL, 28.72 mmol) and a solution of ethyl 4-bromobutanoate (**1**) (6.46 g, 33.81 mmol) in acetonitrile (50 mL). The reaction mixture was stirred at room temperature under argon for 48 h, and then the precipitate was filtered and washed with acetonitrile (10 mL). The filtrate was evaporated; the residue was dissolved in water (70 mL) and a 5% NaOH solution was added until the pH of the medium was 11. The mixture was extracted with ethyl acetate (3 × 50 mL), washed with saturated NaCl (20 mL), dried (Na_2SO_4), and evaporated. The residue (1.7 g) was purified by column chromatography on silica gel with chloroform/methanol (100:2) as eluent to afford **5a** (1.379 g, 34%), oil. ^{1}H NMR ($CDCl_3$, HMDSO) δ 0.30–0.49 (m, 4H), 1.24 (t, J = 7.1 Hz, 3H), 1.59 (tt, J = 3.7, 6.5 Hz, 1H), 1.80 (quintet, J = 7.4 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 2.29 (s, 3H), 2.52 (t, J = 7.4 Hz, 2H), 4.11 (q, J = 7.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 6.7, 14.2, 22.6, 32.3, 38.7, 42.4, 57.3, 66.2, 173.7. HRMS m/z calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$, 186.1494; found, 186.1489.

Ethyl 4-[Cyclobutyl(methyl)amino]butanoate (5b). Compound **5b** was obtained from *N*-methylcyclobutanamine hydrochloride (**30b**) and ethyl 4-bromobutanoate (**1**) by a similar protocol as **5a**; yield 24%. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.25 (t, J = 7.1 Hz, 3H), 1.53–1.70 (m, 2H), 1.75 (quintet, J = 7.4 Hz, 2H), 1.80 (m, 2H), 1.99 (m, 2H), 2.06 (s, 3H), 2.21 (t, J = 7.4 Hz, 2H), 2.31 (t, J = 7.4 Hz, 2H), 2.73 (quintet, J = 7.9 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 13.9, 14.2, 22.5, 27.9, 32.4, 37.7, 53.1, 60.2, 60.5, 173.6. HRMS m/z calcd for $C_{10}H_{22}NO_2$ $[M + H]^+$, 200.1665; found, 200.1651.

Ethyl 4-[Cyclopentyl(methyl)amino]butanoate (5c). Compound **5c** was obtained from *N*-methylcyclopentanamine hydrochloride (**30c**) and ethyl 4-bromobutanoate (**1**) by a similar protocol as **5a**; oil, yield 20%. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.24 (t, J = 7.2 Hz, 3H), 1.40–1.58 (m, 4H), 1.62–1.74 (m, 2H), 1.78–1.89 (m, 4H), 2.29 (s, 3H), 2.32 (t, J = 7.3 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H), 2.76 (m, 1H), 4.12 (q, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 14.2, 21.8, 24.1, 30.1, 32.1, 39.8, 55.0, 60.3, 66.7, 173.4. HRMS m/z calcd for $C_{12}H_{24}NO_2$ $[M + H]^+$, 214.1807; found, 214.1797. Anal. Calcd for $C_{12}H_{23}NO_2$: C 67.57, H 10.87, N 6.57. Found: C 67.63, H 10.91, N 6.46.

Ethyl 4-(Dimethylamino)butanoate (5d). To a solution of ethyl 4-bromobutanoate (**1**) (20.0 g, 102.5 mmol) in anhydrous ethanol (200 mL) was added a 33% (~5.6 M) solution of dimethylamine in ethanol (100 mL, 560 mmol), and the resulting mixture was stirred at ambient temperature for 24 h. The reaction mixture was evaporated, and the residue was dissolved in chloroform (200 mL), washed successively with saturated solutions of $NaHCO_3$ (4 × 50 mL) and NaCl (50 mL), and dried (Na_2SO_4). The solution was evaporated and the residue (12.15 g) was distilled in vacuo at 69–72 °C/13 mmHg to give 10.66 g (65%) of **5d**. ^{1}H NMR [deuterated dimethyl sulfoxide (DMSO-d_6), HMDSO] δ 1.25 (t, J = 7.2 Hz, 3H). 1.78 (quintet, J = 7.4 Hz, 2H), 2.21 (s, 6H), 2.27 (t, J = 7.3 Hz, 2H), 2.32 (t, J = 7.5 Hz, 2H), 4.12 (q, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 14.2, 23.0, 32.1, 45.4, 58.9, 60.2, 173.6. LC-MS (ESI) m/z 160 $[M + H]^+$.

***N*-Butyl-4-ethoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Iodide (6).** A mixture of ethyl 4-(dimethylamino)butanoate (**5d**) (1.26 g, 8.7 mmol) and 1-iodobutane (0.30 mL, 2.63 mmol) in acetonitrile (10 mL) was stirred under argon at room temperature for 24 h and under reflux for 1 h. The solvent was evaporated and the residue was triturated in the ultrasound bath with ether (10 mL). The solid material was filtered and dried in vacuo over P_2O_5 to give **6** (0.597 g, 87%), mp 98–100 °C. ^{1}H NMR (D_2O) δ 0.98 (t, J = 7.4 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H), 1.42 (sextet, J = 7.4 Hz, 2H), 1.77 (m, 2H), 2.10 (m, 2H), 2.54 (t, J = 7.0 Hz, 2H), 3.11 (s, 6H), 3.31–3.38 (m, 4H), 4.21(q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O) δ 12.8, 13.3, 17.4, 19.0, 23.8, 30.2, 50.6 [t, $^1J_{(N,C)}$ = 3.8 Hz], 61.9, 62.6 [unresolved t, $^1J_{(N,C)}$ = 2.8 Hz], 63.9 [t, $^1J_{(N,C)}$ = 2.4 Hz], 174.5. HRMS m/z calcd for $C_{12}H_{26}NO_2$ $[M - I^-]^+$, 216.1964; found, 216.1978. Anal. Calcd for $C_{12}H_{26}INO_2$: C 41.99, H 7.63, N 4.08. Found: C 42.11, H 7.66, N 3.91.

***N*-Benzyl-4-ethoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (7).** Compound **7** was obtained from ethyl 4-(dimethylamino) butanoate (**5d**) and benzylbromide by a similar protocol as **6**; yield 96%, mp 88–92 °C. ^{1}H NMR (D_2O) δ 1.27 (t, J = 7.2 Hz, 3H), 2.21 (m, 2H), 2.53 (t, J = 7.1 Hz, 2H), 3.07 (s, 6H), 3.35 (m, 2H), 4.19 (q, J = 7.2 Hz, 2H), 4.54 (s, 2H), 7.54–7.65 (m, 5H). ^{13}C NMR (D_2O) δ 13.2, 17.6, 30.3, 49.5 [t, $^1J_{(N,C)}$ = 3.8 Hz], 61.9, 62.8 [unresolved t, $^1J_{(N,C)}$ = 3.0 Hz], 68.0 [t, $^1J_{(N,C)}$ = 2.4 Hz], 126.9, 129.1, 130.7, 132.8, 174.5. HRMS m/z calcd for $C_{15}H_{24}NO_2$ $[M - Br^-]^+$, 250.1807; found, 250.1776. Anal. Calcd for $C_{15}H_{24}BrNO_2 \cdot 0.08H_2O$: C 54.31, H 7.34, N 4.22. Found: C 54.31, H 7.37, N 4.22.

***N*-(4-Ethoxy-4-oxobutyl)-*N*,*N*-dimethylcyclopropanaminium Iodide (8).** To a solution of ethyl 4-(cyclopropyl(methyl)amino)-butanoate (**5a**) (1.379 g, 7.44 mmol) in acetonitrile (15 mL) was added iodomethane (2.3 mL, 37.22 mmol). The reaction mixture was stirred at room temperature for 24 h, the solvent was evaporated, and the residue was triturated in an ultrasound bath with ether. The solid material was filtered and dried in vacuo to afford **8** (2.164 g, 84%), mp 87–90 °C ^{1}H NMR (D_2O, DSS) δ 0.96 (m, 2H), 1.22 (m, 2H), 1.27 (t, J = 7.2 Hz, 3H), 2.19 (m, 2H), 2.52 (t, J = 7.1 Hz, 2H), 2.98 (s, 6H), 3.16 (m, 1H), 3.46 (m, 2H), 4.19 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O) δ 1.6, 13.3, 17.7, 30.3, 47.3 [t, $^1J_{(N,C)}$ = 4.2 Hz], 48.8 [t, $^1J_{(N,C)}$ = 3.8 Hz], 61.9, 66.2 [t, $^1J_{(N,C)}$ = 2.9 Hz], 174.6. HRMS m/z calcd for $C_{10}H_{22}NO_2$ $[M - I^-]^+$,

200.1651; found, 200.1653. Anal. Calcd for $C_{11}H_{22}INO_2$: C 40.38, H 6.78, N 4.28. Found: C 40.48, H 6.74, N 4.17.

***N*-(4-Ethoxy-4-oxobutyl)-*N*,*N*-dimethylcyclobutanaminium Iodide (9).** Compound **8** was obtained from ethyl 4-[cyclobutyl(methyl)amino]butanoate (**5b**) and iodomethane by a similar protocol as **8**; yield 100%, mp 95.6−100.5 °C. ^{1}H NMR (D_2O, DSS) δ 1.27 (t, J = 7.2 Hz, 3H), 1.74 (m, 1H), 1.85 (m, 1H), 2.08 (m, 2H), 2.23 (m, 2H), 2.39 (m, 2H), 2.50 (t, J = 7.1 Hz, 2H), 2.98 (s, 6H), 3.23 (m, 2H), 4.10 (quintet, J = 8.7 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O, dioxane) δ 12.4, 13.9, 18.1, 24.3, 31.0, 47.0, 62.4, 62.6, 67.2, 175.3. HRMS m/z calcd for $C_{12}H_{24}NO_2$ $[M - I^-]^+$, 214.1823; found, 214.1807.

***N*-(4-Ethoxy-4-oxobutyl)-*N*,*N*-dimethylcyclopentanaminium Iodide (10).** Compound **10** was obtained from ethyl 4-[cyclopentyl(methyl)amino]butanoate (**5c**) and iodomethane by a similar protocol as **8**; yield 100%, mp 55.9−56.9 °C. ^{1}H NMR (D_2O, DSS) δ 1.29 (t, J = 7.2 Hz, 3H), 1.62−1.94 (m, 6H), 2.01−2.20 (m, 4H), 2.53 (t, J = 7.0 Hz, 2H), 3.05 (s, 6H), 3.34 (m, 2H), 3.98 (quintet, J = 8.1 Hz, 1H), 4.21 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O, dioxane) δ 14.0, 18.1, 24.4, 26.3, 31.0, 48.3 [t, $^1J_{(N,C)}$ = 3.7 Hz], 62.6, 63.6 [unresolved t, $^1J_{(N,C)} \approx$ 2.7 Hz], 75.2, 175.3. LC−MS (ESI) m/z 228.2 $[M - I^-]^+$.

***N*-(Cyclopropylmethyl)-4-ethoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (11).** A mixture of (bromomethyl)cyclopropane (0.500 g, 3.73 mmol) and ethyl 4-(dimethylamino)butanoate (**5d**) (0.500 g, 3.14 mmol) in acetone (3 mL) was stirred under argon at reflux temperature for 8 h and at room temperature overnight. The solvent was evaporated and the residue was triturated in an ultrasound bath with ether (5 mL). The solid material was filtered and dried in vacuo over P_2O_5 to give **11** (0.88 g, 95%), mp 100−103 °C. ^{1}H NMR (D_2O, DSS) δ 0.45 (m, 2H), 0.83 (m, 2H), 1.17 (t quintet, J = 4.9, 7.6 Hz, 1H), 1.27 (t, J = 7.2 Hz, 3H), 2.10 (m, 2H), 2.52 (t, J = 7.1 Hz, 2H), 3.13 (s, 6H), 3.26 (d, J = 7.3 Hz, 2H), 3.41 (m, 2H), 4.19 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O, dioxane) δ 4.5, 4.7, 13.9, 18.2, 31.0, 50.9 [t, $^1J_{(N,C)}$ = 3.9 Hz], 62.6, 63.5 [t, $^1J_{(N,C)}$ = 2.9 Hz], 69.7 [t, $^1J_{(N,C)}$ = 2.3 Hz], 175.3. HRMS m/z calcd for $C_{12}H_{24}NO_2$ $[M - Br^-]^+$, 214.1807; found, 214.1807. Anal. Calcd for $C_{12}H_{24}BrNO_2$: C 48.99, H 8.22, N 4.76. Found: C 48.99, H 8.27, N 4.63.

***N*-(Cyclobutylmethyl)-4-ethoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (12).** A mixture of (bromomethyl)cyclobutane (1.56 g, 10.47 mmol) and ethyl 4-(dimethylamino)butanoate (**5d**) (1.26 g, 8.7 mmol) in acetone (6 mL) was stirred under argon at 50 °C for 48 h. The solvent was evaporated and the residue was triturated in an ultrasound bath with ether (10 mL). The solid material was filtered and dried in vacuo over P_2O_5 to give **12** (0.448 g, 17.5%), mp 97−105 °C. ^{1}H NMR (D_2O, DSS) δ 1.28 (t, J = 7.2 Hz, 3H), 1.63−2.07 (m, 8H), 2.36 (t, J = 7.1 Hz, 2H), 2.74 (septet, J = 7.7 Hz, 1H), 2.89 (s, 6H), 3.15 (m, 2H), 3.24 (d, J = 6.8 Hz, 2H), 4.05 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O) δ 13.2, 17.5, 18.4, 27.3, 29.1, 30.2, 50.5 [t, $^1J_{(N,C)}$ = 3.8 Hz], 61.9, 63.1 [unresolved t, $^1J_{(N,C)} \sim$ 2.8 Hz], 69.1 [unresolved t, $^1J_{(N,C)} \sim$ 1.9 Hz], 174.5. HRMS m/z calcd for $C_{13}H_{26}NO_2$ $[M - Br^-]^+$, 228.1964; found, 228.1964.

4-Ethoxy-*N*-(2-fluoroethyl)-*N*,*N*-dimethyl-4-oxobutan-1-aminium Chloride (13). 2-Fluoroethyl trifluoromethanesulfonate was obtained as described in the literature.[31] To a suspension of poly(4-vinylpyridine) (6.68 g, 60.78 mmol) in anhydrous dichloromethane (100 mL) under argon atmosphere was added trifluoromethanesulphonic anhydride (8.57 g, 30.37 mmol), and the reaction mixture was stirred at ambient temperature for 15 min. To the mixture was added 2-fluoroethanol (1.346 g, 21.30 mmol), and the resulting mixture was stirred for 30 min at ambient temperature and filtered. The precipitate was washed with dichloromethane (30 mL), and the filtrates were combined, washed successively with saturated solutions of $NaHCO_3$ (4 × 50 mL) and NaCl (2 × 50 mL), and dried (Na_2SO_4). The solvent was evaporated at 20 °C/200 mmHg and the obtained crude 2-fluoroethyl trifluoromethanesulfonate was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, HMDSO) δ 4.65 (m, 2H, $^2J_{F,H} \sim$ 47 Hz), 4.66 (m, 2H, $^3J_{F,H} \sim$ 27 Hz).

To a solution of ethyl 4-(dimethylamino)butanoate (**5d**) (2.26 g, 14.2 mmol) in anhydrous dichloromethane (20 mL) at ice bath temperature was added crude 2-fluoroethyl trifluoromethanesulfonate, obtained in the preceding step from 1.346 g (21.30 mmol) of 2-fluoroethanol. The reaction mixture was stirred at ice-bath temperature for 1 h and evaporated. The dark oily residue (5.431 g) was dissolved in water (15 mL), filtered through a pad of cotton, and passed through Amberlite IRA-410 (Cl) ion-exchange resin column (50 mL) slowly (ca. 0.5 mL/min) and eluted with water (control with 2% $AgNO_3$ solution). The eluate was evaporated and the residue was azeotropically dried successively with acetone, 2-propanol, and acetonitrile to give 3.61 g (theoretical amount 3.43 g, 14.2 mmol) of **13**, which was used in the next step without further purification. ^{1}H NMR (D_2O, DSS) δ 1.17 (t, J = 7.2 Hz, 3H); 2.12 (m, 2H); 2.52 (t, J = 7.0 Hz, 2H); 3.20 (s, 6H); 3.46 (m, 2H); 3.80 (m, $^3J_{F,H}$ = 28.2 Hz, 2H); 4.19 (q, J = 7.2 Hz, 2H); 4.97 (m, $^2J_{F,H}$ = 47.3 Hz, 2H).

4-(Ethyldimethylammonio)butanoate (14).[28] A solution of 4-ethoxy-*N*-ethyl-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**2**) (5.00 g, 18.6 mmol) in water (5 mL) was allowed to soak into Amberlite IRA-410 (OH) ion-exchange resin column (75 mL), and the column was stopped. After 0.5 h, the column slowly (ca. 0.2 mL/min) was eluted with water. To the eluate containing expected product (TLC control) was added a small amount of Dowex 50WX8 ion-exchange resin (ca. 0.1−0.2 g) with stirring until pH of the medium changed from ca. 10−11 to 7.5 (ca. 0.5 h, pH-meter). The eluate was filtered and evaporated. The residue was azeotropically dried successively with acetone, 2-propanol, and acetonitrile and then triturated in an ultrasound bath with ether and acetone. The crystals were filtered and dried in vacuo over P_2O_5 to afford highly hygroscopic **14** (2.892 g, 97%). ^{1}H NMR (D_2O, DSS) δ 1.35 (tt, J = 2.0, 7.3 Hz, 3H), 1.99 (m, 2H), 2.27 (t, J = 7.1 Hz, 2H), 3.05 (s, 6H), 3.27 (m, 2H), 3.39 (q, J = 7.3 Hz, 2H). ^{13}C NMR (D_2O) δ 7.3, 18.9, 33.4, 49.9 [t, $^1J_{(N,C)}$ = 4.0 Hz], 59.5 [t, $^1J_{(N,C)}$ = 2.7 Hz], 62.7 [t, $^1J_{(N,C)}$ = 2.8 Hz], 180.7. HRMS m/z calcd for $C_8H_{18}NO_2$ $[M + H]^+$, 160.1338; found, 160.1326. Anal. Calcd for $C_8H_{17}NO_2 \cdot 0.95H_2O$: C 54.49, H 10.80, N 7.94. Found: C 54.51, H 11.14, N 7.88.

4-[Diethyl(methyl)ammonio]butanoate (15).[32] By a similar protocol as **14**, **15** was prepared from 4-ethoxy-*N*,*N*-diethyl-*N*-methyl-4-oxobutan-1-aminium bromide (**3**) to give a highly hygroscopic solid, yield 75%. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.22 (t, J = 7.2 Hz, 6H), 1.67 (m, 2H), 1.81 (t, J = 6.4 Hz, 2H), 2.87 (s, 3H), 3.15 (m, 2H), 3.25 (q, J = 7.2 Hz, 4H). ^{13}C NMR (D_2O) δ 6.9, 18.5, 33.4, 46.7 [t, $^1J_{(N,C)}$ = 4.1 Hz], 56.3 [t, $^1J_{(N,C)}$ = 2.5 Hz], 59.5 [t, $^1J_{(N,C)}$ = 2.7 Hz], 180.7. HRMS m/z calcd for $C_9H_{20}NO_2$ $[M + H]^+$, 174.1494; found, 174.1513. Anal. Calcd for $C_9H_{19}NO_2 \cdot 0.93H_2O$: C 56.89, H 11.07, N 7.37. Found: C 56.91, H 11.73, N 7.26.

4-[(2-Hydroxyethyl)dimethylammonio]butanoate (16). By a similar protocol as **14**, **16** was obtained from 4-ethoxy-*N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**4**) as a highly hygroscopic solid, yield 28%, mp 166−170 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.81−1.92 (m, 2H), 2.20 (t, J = 7.0 Hz, 2H), 3.06 (s, 6H), 3.28−3.35 (m, 2H), 3.35−3.41 (m, 2H), 3.78−3.85 (m, 2H). ^{13}C NMR (D_2O) δ 19.0, 33.4, 51.3, 55.2, 64.7, 64.9 [t, $^1J_{(N,C)}$ = 2.7 Hz], 180.6. LC−MS (ESI) m/z 176 $[M + H]^+$. Anal. Calcd for $C_8H_{17}NO_3 \cdot 1.35H_2O$: C 48.15, H 9.95, N 7.02. Found: C 48.16, H 10.15, N 6.92.

4-(Butyldimethylammonio)butanoate (17). By a similar protocol as **14**, **17** was prepared from *N*-butyl-4-ethoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium iodide (**6**) to give a highly hygroscopic solid, yield 93%, mp 75−100 °C. ^{1}H NMR (D_2O, DSS) δ 0.96 (t, J = 7.4 Hz, 3H), 1.39 (sextet, J = 7.4 Hz, 2H), 1.74 (m, 2H), 1.99 (m, 2H), 2.26 (t, J = 7.1 Hz, 2H), 3.06 (s, 6H), 3.23−3.33 (m, 4H). ^{13}C NMR (D_2O) δ 12.7, 18.9, 23.6, 23.7, 33.4, 50.4 [t, $^1J_{(N,C)}$ = 3.9 Hz], 63.2 [unresolved t, $^1J_{(N,C)}$ = 2.6 Hz], 63.9 [unresolved t, $^1J_{(N,C)}$ = 2.4 Hz], 180.6. HRMS m/z calcd for $C_{10}H_{22}NO_2$ $[M + H]^+$, 188.1651; found, 188.1713. Anal. Calcd for $C_{10}H_{21}NO_2 \cdot 1.01H_2O$: C 58.45, H 11.29, N 6.82. Found: C 58.47, H 11.19, N 6.67.

4-(Benzyldimethylammonio)butanoate (18). By a similar protocol as **14**, **18** was prepared from *N*-benzyl-4-ethoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**7**) to give a highly hygroscopic solid, yield 89%, mp 138−139 °C. ^{1}H NMR (D_2O, DSS) δ 2.12 (m, 2H), 2.28 (t, J = 7.2 Hz, 2H), 3.04 (s, 6H), 3.31 (m, 2H), 4.50 (s, 2H), 7.55−7.64 (m, 5H). ^{13}C NMR (D_2O) δ 19.1, 33.5, 49.4 [t, $^1J_{(N,C)}$ = 3.8 Hz], 63.6 [t, $^1J_{(N,C)}$ = 2.8 Hz], 67.8 [unresolved t, $^1J_{(N,C)}$ = 2.6 Hz], 127.0, 129.1,

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130.7, 132.8, 180.6. HRMS m/z calcd for $C_{13}H_{20}NO_2$ $[M+H]^+$, 222.1494; found, 222.1519. Anal. Calcd for $C_{13}H_{19}NO_2 \cdot 0.61H_2O$: C 67.22, H 8.77, N 6.03. Found: C 67.22, H 9.10, N 5.97.

4-(Cyclopropyldimethylammonio)butanoate (19). By a similar protocol as **14**, **19** was obtained from *N*-(4-ethoxy-4-oxobutyl)-*N*,*N*-dimethylcyclopropanaminium iodide (**8**) as a highly hygroscopic solid, yield 74%, mp 174.5−175.5 °C. ^{1}H NMR (D_2O, DSS) δ 0.95 (m, 2H), 1.20 (m, 2H), 2.11 (m, 2H), 2.27 (t, J = 7.2 Hz, 2H), 2.96 (s, 6H), 3.14 (m, 1H), 3.41 (m, 2H). ^{13}C NMR (D_2O, dioxane) δ 1.7, 19.4, 33.7, 47.4, 48.8, 67.0, 180.9. LC−MS (ESI) m/z: 172 $[M + H]^+$. Anal. Calcd for $C_9H_{17}NO_2 \cdot 0.5H_2O$: C 59.97, H 10.07, N 7.77. Found: C 60.06, H 10.70, N 7.70.

4-(Cyclobutyldimethylammonio)butanoate (20). By a similar protocol as **14**, **20** was obtained from *N*-(4-ethoxy-4-oxobutyl)-*N*,*N*-dimethylcyclobutanaminium iodide (**9**) as a highly hygroscopic solid, yield 91%. ^{1}H NMR (D_2O, DSS) δ 1.71 (tq, J = 8.0, 10.4 Hz, 1H), 1.83 (tq, J = 2.4, 10.4 Hz, 1H), 1.98 (m, 2H), 2.21 (m, 2H), 2.23 (t, J = 7.2 Hz, 2H), 2.37 (m, 2H), 2.95 (s, 6H), 3.17 (m, 2H), 4.07 (tt, J = 7.8, 9.7 Hz, 1H). ^{13}C NMR (D_2O, dioxane) δ 12.4, 19.6, 24.3, 34.2, 46.8, 62.9, 67.2, 181.3. HRMS m/z calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$, 186.1494; found, 186.1506. Anal. Calcd for $C_{10}H_{19}NO_2 \cdot 1.25H_2O$: C 57.81, H 10.43, N 6.74. Found: C 57.79, H 10.51, N 6.57.

4-(Cyclopentyldimethylammonio)butanoate (21). By a similar protocol as **14**, **21** was obtained from *N*-(4-ethoxy-4-oxobutyl)-*N*,*N*-dimethylcyclopentanaminium iodide (**10**) as a highly hygroscopic solid, yield 92%, mp 151.6−153.6 °C. ^{1}H NMR (D_2O) δ 1.45−1.57 (m, 2H), 1.57−1.76 (m, 4H), 1.89 (m, 4H), 2.10 (t, J = 7.1 Hz, 2H), 2.86 (s, 6H), 3.12 (m, 2H), 3.79 (quintet, J = 8.2 Hz, 1H). ^{13}C NMR (D_2O, dioxane) δ 19.6, 24.4, 26.3, 34.2, 48.2 [t, $^1J_{(N,C)}$ = 3.8 Hz], 64.2, 75.2, 181.4. HRMS m/z calcd for $C_{11}H_{22}NO_2$ $[M + H]^+$, 200.1651; found, 200.1635. Anal. Calcd for $C_{11}H_{21}NO_2 \cdot 1.01H_2O$: C 60.75, H 10.67, N 6.44. Found: C 60.73, H 11.44, N 6.43.

4-[(Cyclopropylmethyl)dimethylammonio]butanoate (22). By a similar protocol as **14**, **22** was obtained from *N*-(cyclopropylmethyl)-4-ethoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**11**) as a highly hygroscopic solid, yield 53%, mp 169−174 °C. ^{1}H NMR (D_2O, DSS) δ 0.45 (m, 2H), 0.81 (m, 2H), 1.16 (t quintet, J = 4.9, 7.6 Hz, 1H), 2.01 (m, 2H), 2.27 (t, J = 7.2 Hz, 2H), 3.12 (s, 6H), 3.25 (d, J = 7.3 Hz, 2H), 3.36 (m, 2H). ^{13}C NMR (D_2O, dioxane) δ 4.4, 4.7, 19.7, 34.3, 50.9 [t, $^1J_{(N,C)}$ = 3.9 Hz], 64.2 [unresolved t, $^1J_{(N,C)} \sim$ 2.6 Hz], 69.7 [unresolved t, $^1J_{(N,C)} \sim$ 2.3 Hz], 181.4. HRMS m/z calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$, 186.1494; found, 186.1497. Anal. Calcd for $C_{10}H_{19}NO_2 \cdot 0.34H_2O$: C 62.76, H 10.36, N 7.32. Found: C 62.75, H 10.70, N 7.26.

4-[(Cyclobutylmethyl)dimethylammonio]butanoate (23). By a similar protocol as **14**, **23** was obtained from 4-ethoxy-*N*-ethyl-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**12**) as a highly hygroscopic solid, yield 78%, mp 141−147 °C. ^{1}H NMR (D_2O) δ 1.81−2.09 (m, 6H), 2.20 (m, 2H), 2.29 (t, J = 7.2 Hz, 2H), 2.92 (septet, J = 7.7 Hz, 1H), 3.06 (s, 6H), 3.28 (m, 2H), 3.40 (d, J = 6.8 Hz, 2H). ^{13}C NMR (D_2O, dioxane) δ 19.1, 19.7, 28.0, 29.8, 34.2, 51.1 [t, $^1J_{(N,C)}$ = 3.8 Hz], 64.5 [unresolved t, $^1J_{(N,C)}$ = 2.6 Hz], 69.9 [unresolved t, $^1J_{(N,C)}$ = 2.1 Hz], 181.4. HRMS m/z calcd for $C_{11}H_{22}NO_2$ $[M + H]^+$, 200.1651; found, 200.1626. Anal. Calcd for $C_{11}H_{21}NO_2 \cdot 0.98H_2O$: C 60.90, H 10.67, N 6.46. Found: C 60.89, H 11.44, N 6.40.

3-Carboxy-*N*-(2-fluoroethyl)-*N*,*N*-dimethylpropan-1-aminium chloride (24).[27] To a solution of crude 4-ethoxy-*N*-(2-fluoroethyl)-*N*,*N*-dimethyl-4-oxobutan-1-aminium chloride (**13**) (3.61 g, theoretically 14.2 mmol) in dioxane (10 mL) was added concentrated HCl (5 mL), and the reaction mixture was stirred at 40 °C for 24 h. The mixture was evaporated, and to the residue (3.02 g) was added concentrated HCl (15 mL). The mixture was stirred at 70 °C for 2 h and then evaporated again. The residue was triturated with acetonitrile in an ultrasound bath, and then the mixture was decanted and the crystalline solid was washed with acetone. After drying in vacuo over P_2O_5, **24** (2.125 g, 70% on **5d**) was obtained, mp 151.1−153.5 °C. ^{1}H NMR (D_2O, DSS) δ 2.05−2.16 (m, 2H), 2.52 (t, J = 7.0 Hz, 2H), 3.20 (s, 6H), 3.44−3.51 (m, 2H), 3.80 [m, $^3J_{(F,H)}$ = 28.3 Hz, 2H], 4.98 [m, $^2J_{(F,H)}$ = 47.3 Hz, 2H]. ^{13}C NMR (D_2O) δ 17.5, 29.9, 51.2 [td, $^1J_{(N,C)}$ = 3.1 Hz, $^4J_{(F,C)}$ = 3.4 Hz], 63.6 [td, $^1J_{(N,C)}$ = 3.3 Hz, $^2J_{(F,C)}$ = 19.1 Hz], 64.1 [unresolved q, $^1J_{(N,C)}$ and $^4J_{(F,C)} \sim$ 2.7 Hz], 77.5 [d, $^1J_{(F,C)}$ = 168.5 Hz], 176.3. HRMS m/z calcd for $C_8H_{17}FNO_2$ $[M + H]^+$, 178.1243; found, 178.1253. Anal. Calcd for $C_8H_{17}ClFNO_2 \cdot 0.25H_2O$: C 44.04, H 8.08, N 6.42. Found: C 44.06, H 8.00, N 6.33. Cl^- counterion titration: calcd 16.59%, found 16.52%.

1-(3-Carboxypropyl)pyrrolidin-1-ium Chloride (25). Compound **25** was synthesized according to a literature procedure;[33] mp 116−119 °C. ^{1}H NMR (D_2O, DSS) δ 1.95−2.07 (m, 4H), 2.09−2.21 (m, 2H), 2.52 (t, J = 7.2 Hz, 2H), 3.10 (m, 2H), 3.25 (m, 2H), 3.69 (m, 2H). ^{13}C NMR (D_2O) δ 20.6, 22.5, 30.3, 53.8, 54.0, 176.6. HRMS m/z calcd for $C_8H_{16}NO_2$ $[M - Cl^-]^+$, 158.1181; found, 158.1253. HRMS m/z calcd for $C_8H_{15}NO_2Na$ $[M - HCl + Na^+]^+$, 180.1000; found, 180.1063. Anal. Calcd for $C_8H_{16}ClNO_2 \cdot 0.25H_2O$: C 48.49, H 8.39, N 7.07. Found: C 48.47, H 8.20, N 6.94. Cl^- counterion titration: calcd 18.31%, found 18.72%.

1-(3-Carboxypropyl)-1-methylpyrrolidin-1-ium Chloride (26). Compound **26** was synthesized according to a literature procedure.[33] ^{1}H NMR (D_2O, DSS) δ 2.11 (m, 2H), 2.23 (m, 4H), 2.52 (t, J = 7.0 Hz, 2H), 3.08 (s, 3H), 3.39 (m, 2H), 3.54 (m, 4H). ^{13}C NMR (D_2O) δ 18.6, 21.2, 30.1, 47.9 [t, $^1J_{(N,C)}$ = 3.9 Hz], 62.8 [t, $^1J_{(N,C)}$ = 3.1 Hz], 64.3 [t, $^1J_{(N,C)}$ = 2.9 Hz], 176.4. HRMS m/z calcd for $C_9H_{18}NO_2$ $[M - Cl^-]^+$, 172.1338; found, 172.1343. Anal. Calcd for $C_9H_{18}ClNO_2 \cdot 0.03H_2O$: C 51.91, H 8.74, N 6.73. Found: C 51.90, H 8.82, N 6.57. Cl^- counterion titration: calcd 17.07%, found 16.96%.

***tert*-Butyl Cyclopropylcarbamate (28a).** Compound **28a** was prepared as described in ref 34. To a solution of cyclopropanamine (**27a**) (7.0 mL, 101 mmol) in dichloromethane (200 mL) under argon atmosphere at ice-bath temperature was added a solution of di-*tert*-butyl dicarbonate (24.23 g, 111.1 mmol) in dichloromethane (100 mL). The reaction mixture was stirred at room temperature for 1 h, washed with water (2 × 50 mL) and saturated NaCl (2 × 50 mL), and dried (Na_2SO_4). The solvent was evaporated to afford **28a** (15.5 g, quant), which was used in further steps without additional purification; mp 62.5−64 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ 0.47 (m, 2H), 0.67 (m, 2H), 1.44 (s, 9H), 2.52 (m, 1H), 4.72 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 6.7, 22.8, 28.3, 79.3, 156.6. LC−MS (ESI) m/z: 158 $[M + H]^+$. Anal. Calcd for $C_8H_{15}NO_2$: C 61.12, H 9.62, N 8.91. Found: C 60.98, H 9.60, N 8.79.

***tert*-Butyl Cyclobutylcarbamate (28b).** Compound **28b** was obtained from cyclobutanamine (**27b**) as described in ref 35; yield 91%. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.42 (s, 9H), 1.55−1.72 (m, 2H), 1.80 (m, 2H), 2.29 (m, 2H), 4.09 (m, 1H), 4.68 (br s, 1H).

***tert*-Butyl Cyclopentylcarbamate (28c).** Compound **28c** was obtained from cyclopentanamine (**27c**) by a similar protocol as **28a**. The obtained solid product was crystallized from petroleum ether, yield 77%, mp 74.0−75.8 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.35 (m, 2H), 1.43 (s, 9H), 1.50−1.70 (m, 4H), 1.92 (m, 2H), 3.91 (m, 1H), 4.45 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 23.5, 28.4, 33.3, 52.3, 78.9, 155.5. HRMS m/z calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$, 186.1494; found, 186.1519. Anal. Calcd for $C_{10}H_{19}NO_2 \cdot 0.1C_6H_{12}$: C 65.67, H 10.60, N 7.22. Found: C 65.83, H 10.48, N 7.13.

***tert*-Butyl Cyclopropyl(methyl)carbamate (29a).** To a solution of *tert*-butyl cyclopropylcarbamate (**28a**) (5.988 g, 38.1 mmol) in *N*,*N*-dimethylformamide (DMF; 15 mL) under argon atmosphere at ice-bath temperature was added 60% sodium hydride in mineral oil (1.57 g, 39.23 mmol) portionwise. The reaction mixture was stirred at room temperature for 1 h, and then iodomethane (2.5 mL, 40.0 mml) was added and the stirring was continued overnight. The mixture was poured into ice water (150 mL), extracted with ether (3 × 50 mL), washed with saturated NaCl (50 mL), and dried (Na_2SO_4). The solvent was evaporated and the residue (5.899 g) was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (95:5) as eluent to afford **29a** (3.477 g, 53%) as an oil. ^{1}H NMR ($CDCl_3$, HMDSO) δ 0.58 (m, 2H), 0.70 (m, 2H), 1.45 (s, 9H), 2.49 (tt, J = 3.8, 4.9 Hz, 1H), 2.82 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 7.8, 28.4, 30.2, 34.7, 79.2, 157.0. LC−MS (ESI) m/z 172 $[M + H]^+$

***tert*-Butyl Cyclobutyl(methyl)carbamate (29b).** Compound **29b** was obtained from *tert*-butyl cyclobutylcarbamate (**28b**) by a similar protocol as **29a**. The crude product was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (9:1) as eluent to afford known[35] **29b** as an oil, yield 46%. ^{1}H NMR

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($CDCl_3$, HMDSO) δ 1.44 (s, 9H), 1.50−1.67 (m, 2H), 2.00−2.14 (m, 4H), 2.78 (s, 3H), 4.42 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 14.5, 28.1, 28.5, 29.1, 50.7, 79.2, 155.6.

***tert*-Butyl Cyclopentyl(methyl)carbamate (29c).** Compound **29c** was obtained from *tert*-butyl cyclopentylcarbamate (**28c**) by a similar protocol as **29a**; oil, yield 36%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.40−1.89 (m, 8H), 2.71 (s, 3H), 4.44 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 24.1, 28.0, 28.4, 28.5, 56.4, 79.1, 155.9. LC−MS (ESI) m/z 144.1, $[M - {}^t\text{Bu} + 2H]^+$, 185.1 $[M - {}^t\text{Bu} + CH_3CN + 2H]^+$.

***N*-Methylcyclopropanamine Hydrochloride (30a).** To methanol (50 mL) at ice-bath temperature was added slowly acetyl chloride (7.7 mL, 101.5 mmol). The obtained solution was stirred for 15 min and then *tert*-butyl cyclopropyl(methyl)carbamate (**29a**) (3.477 g, 20.3 mmol) was added. The reaction mixture was stirred at room temperature for 4 h, solvents were evaporated, and the residue was dried in vacuo to give **30a** (2.104 g, 96%) as white crystals, mp 60.3−86.3 °C. ^{1}H NMR (D_2O, DSS) δ 0.82−0.96 (m, 4H), 2.75 (m, 1H), 2.79 (s, 3H). ^{13}C NMR (D_2O, dioxane) δ 3.5, 32.1, 33.7. LC−MS (ESI) m/z 72.0 $[M - Cl^-]^+$. Anal. Calcd for $C_4H_{10}ClN{\cdot}0.39H_2O$: C 41.92, H 9.48, N 12.22. Found: C 41.95, H 10.15, N 12.06.

***N*-Methylcyclobutanamine Hydrochloride (30b).[35]** Compound **30b** was obtained from *tert*-butyl cyclobutyl(methyl)carbamate (**29b**) by a similar protocol as **30a**; yield 90%, white hygroscopic crystals. ^{1}H NMR (D_2O, DSS) δ 1.80−1.96 (m, 2H), 2.14 (m, 2H), 2.33 (m, 2H), 2.60 (s, 3H), 3.72 (quintet, J = 8.1 Hz, 1H). ^{13}C NMR (D_2O, dioxane) δ 14.7, 26.3, 30.4, 53.4. HRMS m/z calcd for $C_5H_{12}N$ $[M - Cl^-]^+$, 86.0970; found, 86.0975.

***N*-Methylcyclopentanamine Hydrochloride (30c).** Compound **30c** was obtained from *tert*-butyl cyclopentyl(methyl)carbamate (**29c**) by a similar protocol as **30a**; yield 100%, white hygroscopic crystals, mp 75−80 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47−1.75 (m, 2H), 1.75−2.19 (m, 6H), 2.65 (t, J = 5.4 Hz, 3H), 3.36 (m, 1H), 9.40 (br s, 2H). ^{13}C NMR (D_2O) δ 23.5, 29.0, 31.3, 60.6. LC−MS (ESI) m/z 100.0 $[M - Cl^-]^+$. Anal. Calcd for $C_6H_{14}ClN{\cdot}0.06H_2O$: C 52.71, H 10.41, N 10.25. Found: C 52.70, H 10.49, N 10.20.

3-Carboxy-*N*,*N*-dimethyl-1-propanaminium Chloride (32). Compound **32** was obtained from 4-aminobutanoic acid (**31**) in 69−83% yield as described in the literature .[14]

4-Methoxy-*N*,*N*-dimethyl-4-oxo-1-butanaminium Chloride (33a). To a solution of 3-carboxy-*N*,*N*-dimethyl-1-propanaminium chloride (**32**) (45.93 g, 0.27 mol) in anhydrous methanol (300 mL) at −10−0 °C was added slowly thionyl chloride (55 mL, 0.76 mol), and the temperature of the reaction mixture was allowed to rise to room temperature during ca. 1 h. The mixture was stirred at 40−50 °C for 3 h and evaporated. The residue was dissolved in acetone (110 mL) and precipitated by addition of diethyl ether (400 mL). The solid was filtered, washed with ether, and once more dissolved in acetone (110 mL), followed by precipitation with ether (400 mL). The precipitate was filtered, washed with ether, and dried to give 38.4 g (77%) of **33a**. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.91 (quintet, J = 7.7 Hz, 2H), 2.43 (t, J = 7.74 Hz, 2H), 2.71 (d, J = 4.9 Hz, 6H), 2.98−3.06 (m, 2H), 3.61 (s, 3H), 10.76 (br s, 1H). ^{13}C NMR (D_2O) δ 19.3, 30.2, 42.6, 52.3, 56.6, 175.2. LC−MS (ESI) m/z 146.2 $[M - Cl^-]^+$. Anal. Calcd for $C_7H_{16}ClNO_2{\cdot}0.55H_2O$: C 43.89, H 9.00, N 7.31. Found: C 43.91, H 8.75, N 7.52.

Methyl 4-(Dimethylamino)butanoate (33b). A suspension of 4-methoxy-*N*,*N*-dimethyl-4-oxo-1-butanaminium chloride (**33a**) (5.44 g, 0.03 mol) and anhydrous K_2CO_3 (5.52 g, 0.04 mol) in dichloromethane (70 mL) was vigorously stirred at room temperature for 24 h. The precipitate was filtered and washed with dichloromethane, and the filtrate was evaporated. The residue was distilled at 32−35 °C/3−4 mmHg to give 2.88 g (66%) of **33b**. ^{1}H NMR ($CDCl_3$) δ 1.72 (quintet, J = 7.4 Hz, 2H), 2.14 (s, 6H), 2.21 (t, J = 7.3 Hz, 2H), 2.28 (t, J = 7.5 Hz, 2H), 3.60 (s, 3H). ^{13}H NMR ($CDCl_3$) δ 22.8, 31.7, 45.3, 51.4, 58.7, 173.9. GC−MS (EI, 70 eV), m/z (I_{rel}, %) 58.1 $[C_3H_8N]^+$ (100), 114.1 $[M - OCH_3]^+$ (16), 145.1 M^+ (9).

4-Methoxy-*N*,*N*-dimethyl-4-oxo-*N*-propylbutan-1-aminium Bromide (34). A mixture of 4-methoxy-*N*,*N*-dimethyl-4-oxo-1-butanaminium chloride (**33a**) (9.08 g, 50 mmol), 1-bromopropane (12.3 g, 100 mmol), and anhydrous potassium carbonate (13.8 g, 100 mmol) in acetone (65 mL) was stirred at room temperature for 5 days. The reaction mixture was evaporated and the residue was azeotropically dried with 2-propanol (50 mL), mixed with 2-propanol (100 mL), and kept in a refrigerator overnight. The precipitated solid (18.7 g) was filtered off, the filtrate was evaporated, and the residue (14.26 g) was crystallized from acetone/ether and acetone/ethyl acetate mixtures and dried in vacuo over P_2O_5 to afford **34** (9.16 g, 68%), mp 81−85 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ 0.90 (t, J = 7.3 Hz, 3H), 1.67 (m, 2H), 1.91 (m, 2H), 2.41 (t, J = 7.2 Hz, 2H), 3.01 (s, 6H), 3.17−3.28 (m, 4H), 3.62 (s, 3H). ^{13}C NMR (D_2O) δ 9.8, 15.5, 17.4, 29.9, 50.6 [t, $^1J_{(N,C)}$ = 3.9 Hz], 52.4, 62.6 [t, $^1J_{(N,C)}$ = 2.9 Hz], 65.5 [t, $^1J_{(N,C)}$ = 2.4 Hz], 175.0. LC−MS (ESI) m/z 181.1 $[M - Br^-]^+$.

***N*-(4-Methoxy-4-oxobutyl)-*N*,*N*-dimethylprop-2-en-1-aminium Bromide (35).** A mixture of methyl 4-(dimethylamino)-butanoate hydrochloride (**33a**) (1.00 g, 5.50 mmol), 3-bromoprop-1-ene (0.95 mL, 11.0 mmol), and $NaHCO_3$ (0.51 g, 6.06 mmol) in acetone (5 mL) was stirred for 13 days at ambient temperature in a closed vessel. The reaction mixture was evaporated, and the residue was mixed with 2-propanol (20 mL) and kept in a freezer overnight. The precipitate (0.38 g of inorganic salts) was filtered off, the filtrate was evaporated, and the residue was sedimented from acetone (6 mL) and diethyl ether (15 mL) and dried in vacuo over P_2O_5 to afford 1.30 g (88%) of **35**, mp 90−93 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.95 (m, 2H); 2.41 (t, J = 7.2 Hz, 2H), 2.98 (s, 6H), 3.22 (m, 2H), 3.62 (s, 3H), 3.95 (d, J = 7.3 Hz, 2H); 5.63 (dd, J = 1.4, 17.4 Hz, 1H), 5.63 (dd, J = 1.4, 9.7 Hz, 1H), 6.03 (tdd, J = 7.3, 9.7, 17.4 Hz, 1H). ^{13}C NMR (D_2O) δ 17.4, 29.9, 50.0 [t, $^1J_{(N,C)}$ = 4.1 Hz], 52.3, 62.5 [t, $^1J_{(N,C)}$ = 3.1 Hz], 66.3 [t, $^1J_{(N,C)}$ = 2.9 Hz], 124.1, 129.0, 175.0. LC−MS (ESI) m/z 186.1 $[M - Br^-]^+$. Anal. Calcd for $C_{10}H_{20}BrNO_2{\cdot}0.42H_2O$: C 43.88, H 7.67, N 5.12. Found: C 43.88, H 7.62, N 5.02.

***N*-Isopropyl-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (36).** A mixture of methyl 4-(dimethylamino)-butanoate (**33b**) (3.00 g, 20.66 mmol) and 2-bromopropane (19 mL, 206.6 mmol) in acetonitrile (25 mL) was stirred in a closed vessel for 3 days at ambient temperature and 1 day at 65 °C. The reaction mixture was evaporated, and the white solid was triturated with diethyl ether and dried to afford 4.825 g (87%) of **36**, mp 134−137 °C. ^{1}H NMR (D_2O, DSS) δ 1.40 (d, J = 6.6 Hz, 6H), 2.10 (m, 2H), 2.53 (t, J = 7.1 Hz, 2H), 3.01 (s, 6H), 3.33 (m, 2H), 3.73 (s, 3H), 3.76 (septet, J = 6.6 Hz, 1H). ^{13}C NMR (D_2O) δ 15.4, 17.2, 29.9, 47.3 [t, $^1J_{(N,C)}$ = 3.9 Hz], 52.3, 61.4 [t, $^1J_{(N,C)}$ = 2.9 Hz], 64.9, 175.0. LC−MS (ESI) m/z: 188 $[M - Br^-]^+$. Anal. Calcd for $C_{10}H_{22}BrNO_2{\cdot}0.22H_2O$: C 44.13, H 8.31, N 5.15. Found: C 44.13, H 8.47, N 5.03.

4-Methoxy-*N*,*N*-dimethyl-4-oxo-*N*-(prop-2-yn-1-yl)butan-1-aminium Bromide (37). A mixture of methyl 4-(dimethylamino)-butanoate (**33b**) (1.928 g, 13.3 mmol) and 3-bromoprop-1-yne (1.58 g, 13.3 mmol) in acetone (6 mL) was stirred for 3 days at ambient temperature in a closed vessel. The reaction mixture was evaporated, and the solid residue was azeotropically dried with 2-propanol and sedimented twice from 2-propanol (7 mL) and ethyl acetate (40 mL). The obtained hygroscopic white crystals were dried in vacuo over P_2O_5 to afford 2.54 g (72%) of **37**, mp 103−109 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.95 (m, 2H), 2.43 (t, J = 7.2 Hz, 2H), 3.08 (s, 6H), 3.36 (m, 2H), 3.62 (s, 3H), 4.07 (t, J = 2.5 Hz, 1H), 4.38 (d, J = 2.5 Hz, 2H). ^{13}C NMR (D_2O) δ 17.6, 29.8, 50.4, 52.3, 54.1, 62.8, 70.7, 81.5, 174.9. LC−MS (ESI) m/z 184 $[M - Br^-]^+$. IR (film) 3013, 2953, 2931, 2122, 1736, 1636, 1482, 1439, 1375, 1287, 1206, 1087, 1037 cm^{-1}. Anal. Calcd for $C_{10}H_{18}BrNO_2{\cdot}0.16H_2O$: C 44.98, H 6.91, N 5.25. Found: C 44.97, H 6.88, N 5.17.

***N*-(Chloromethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Chloride (38).** A solution of methyl 4-(dimethylamino)-butanoate (33b) (3.39 g, 23.35 mmol) in anhydrous dichloromethane (50 mL) was stirred for 3 days at ambient temperature and 3 days under reflux. The reaction mixture was evaporated and the oily solid was triturated with diethyl ether (30 mL) in an ultrasound bath. The crystals were filtered, washed with diethyl ether, and dried in vacuo over P_2O_5 to afford 2.36 g (44%) of **38**. ^{1}H NMR (D_2O, DSS) δ 2.12 (m, 2H), 2.56 (t, J = 7.0 Hz, 2H), 3.24 (s, 6H), 3.52 (m, 2H), 3.73 (s, 3H), 5.19 (s, 2H). ^{13}C NMR (D_2O) δ 17.3, 29.7, 49.4, 52.4, 61.8, 68.6, 174.8. LC−MS (ESI) m/z 194.1 $[M - Cl^-]^+$. Anal. Calcd for $C_8H_{17}Cl_2NO_2{\cdot}0.67H_2O$: C 39.67, H 7.63, N 5.78. Found: C 39.67, H 7.87, N 5.68.

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***N*-(Bromomethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (39).** A solution of methyl 4-(dimethylamino)-butanoate (**33b**) (1.45 g, 10 mmol) in dibromomethane (10 mL) was stirred at room temperature overnight. The precipitated solid was filtered and dried in vacuo over P_2O_5 to give 2.84 g (89%) of **39**. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.95 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 3.18 (s, 6H), 3.44 (m, 2H), 3.63 (s, 3H), 5.38 (s, 2H). ^{13}C NMR (D_2O) δ 17.5, 29.7, 50.4, 52.3, 55.9, 62.8, 174.8. LC−MS (ESI) m/z 238.0 [M − Br^-]$^+$.

***N*-(Iodomethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Iodide (40).** Compound **40** was obtained from methyl 4-(dimethylamino)butanoate (**33b**) and diiodomethane by a similar protocol as **39**; yield 70%. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.93 (m, 2H), 2.44 (t, J = 7.2 Hz, 2H), 3.15 (s, 6H), 3.39 (m, 2H), 3.63 (s, 3H), 5.19 (s, 2H). ^{13}C NMR (D_2O) δ 17.8, 29.6, 30.7, 51.7, 52.4, 64.0, 174.8. LC−MS (ESI) m/z 286.0 [M − I^-]$^+$.

4-Methoxy-*N*-(methoxymethyl)-*N*,*N*-dimethyl-4-oxobutan-1-aminium Chloride (41). Compound **41** was obtained from methyl 4-(dimethylamino)butanoate (**33b**) and chloro(methoxy)methane by a similar protocol as **39** as an oil after evaporation of volatiles, yield 71%. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.92 (m, 2H), 2.41 (t, J = 7.2 Hz, 2H), 2.96 (s, 6H), 3.23 (m, 2H), 3.60 (s, 3H), 3.62 (s, 3H), 4.62 (s, 2H). ^{13}C NMR (D_2O) δ 17.2, 30.0, 47.0 [t, $^1J_{(N,C)}$ = 3.6 Hz], 52.3, 59.9 [t, $^1J_{(N,C)}$ = 2.6 Hz], 60.8, 91.8 [t, $^1J_{(N,C)}$ = 2.8 Hz], 174.9. LC−MS (ESI) m/z 190.1 [M − Cl^-]$^+$.

***N*-(2-Chloroethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (42).** To a solution of methyl 4-(dimethylamino)-butanoate (**33b**) (4.00 g, 27.6 mmol) in acetonitrile (50 mL) was added 1-bromo-2-chloroethane (23 mL, 276 mmol), and the obtained mixture was stirred in a closed vessel at 65 °C for 5 days. The reaction mixture was evaporated, and the white solid residue was washed with diethyl ether and dried in vacuo over P_2O_5 to give 7.477 g (94%) of **42**, mp 80−90 °C. ^{1}H NMR (D_2O, DSS) δ 2.13 (m, 2H), 2.54 (t, J = 7.0 Hz, 2H), 3.20 (s, 6H), 3.45 (m, 2H), 3.73 (s, 3H), 3.80 (t, J = 6.7 Hz, 2H), 4.03 (t, J = 6.7 Hz, 2H). ^{13}C NMR (D_2O) δ 17.5, 29.8, 35.3, 51.2 [t, $^1J_{(N,C)}$ = 3.5 Hz], 52.3, 63.7 [t, $^1J_{(N,C)}$ = 2.3 Hz], 63.8 [t, $^1J_{(N,C)}$ = 2.9 Hz], 175.8. LC−MS (ESI) m/z 208 [M − Br^-]$^+$. Anal. Calcd for $C_9H_{19}BrClNO_2$: C 37.45, H 6.64, N 4.85. Found: C 37.53, H 6.65, N 4.74.

***N*-(2-Bromoethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium Bromide (43).** A mixture of methyl 4-(dimethylamino)-butanoate (**33b**) (1.03 g, 7.1 mmol) and 1,2-dibromoethane (12.36 g, 65.8 mmol) was stirred for 10 days at room temperature in a closed vessel. Ethyl acetate (15 mL) was added to the reaction mixture, and the precipitate was filtered, washed with ethyl acetate, and dried in vacuo over P_2O_5 to afford 2.27 g (96%) of **43**, mp 105−109 °C. ^{1}H NMR (D_2O, DSS) δ 2.12 (m, 2H), 2.54 (t, J = 7.0 Hz, 2H), 3.17 (s, 6H), 3.42 (m, 2H), 3.73 (s, 3H), 3.75−3.88 (m, 4H). ^{13}C NMR (D_2O) δ 17.4, 20.5, 29.7, 50.8 [t, $^1J_{(N,C)}$ = 3.6 Hz], 52.3, 63.3 [t, $^1J_{(N,C)}$ = 2.5 Hz], 63.6 [t, $^1J_{(N,C)}$ = 2.3 Hz], 174.9. LC−MS (ESI) m/z 252 [M − Br^-]$^+$. Anal. Calcd for $C_9H_{19}Br_2NO_2$: C 32.46, H 5.75, N 4.21. Found: 32.51, H 5.68, N 4.11.

4-[Dimethyl(propyl)ammonio]butanoate (44). Compound **44** was obtained from 4-methoxy-*N*,*N*-dimethyl-4-oxo-*N*-propylbutan-1-aminium bromide (**34**) as hygroscopic crystals by a similar protocol as **14**; yield 86%. ^{1}H NMR (DMSO-d_6, HMDSO) δ 0.89 (t, J = 7.4 Hz, 3H), 1.63−1.76 (m, 4H), 1.80 (t, J = 6.4 Hz, 2H), 2.97 (s, 6H), 3.13−3.25 (m, 4H). ^{13}C NMR (D_2O) δ 9.7, 15.5, 18.9, 33.4, 50.4 [t, $^1J_{(N,C)}$ = 3.9 Hz], 63.3 [t, $^1J_{(N,C)}$ = 2.7 Hz], 65.4 [t, $^1J_{(N,C)}$ = 2.5 Hz], 180.7. HRMS m/z calcd for $C_9H_{20}NO_2$ [M + H]$^+$, 174.1494; found, 174.1488. Anal. Calcd for $C_9H_{19}NO_2\cdot1.17H_2O$: C 55.63, H 11.07, N 7.21. Found: C 55.61, H 10.83, N 7.11.

4-(Allyldimethylammonio)butanoate (45). Compound **45** was obtained from *N*-(4-methoxy-4-oxobutyl)-*N*,*N*-dimethylprop-2-en-1-aminium bromide (**35**) as hygroscopic crystals by a similar protocol as **14**; yield 81%. ^{1}H NMR (D_2O, DSS) δ 1.71−1.83 (m, 4H), 2.95 (s, 6H), 3.20 (m, 2H), 3.92 (d, J = 7.2 Hz, 2H), 5.60 (d, J = 10.4 Hz, 1H), 5.61 (d, J = 16.8 Hz, 1H), 6.10 (tdd, J = 7.2, 10.4, 16.8 Hz, 1H). ^{13}C NMR (D_2O) δ 18.9, 33.4, 49.9 [t, $^1J_{(N,C)}$ = 4.1 Hz], 63.2 [t, $^1J_{(N,C)}$ = 2.9 Hz], 66.2 [t, $^1J_{(N,C)}$ = 2.8 Hz], 124.2, 128.9, 180.6. HRMS m/z calcd for $C_9H_{18}NO_2$ [M + H]$^+$, 172.1338; found, 172.1331. Anal. Calcd for $C_9H_{17}NO_2\cdot0.53H_2O$: C 59.79, H 10.07, N 7.75. Found: C 59.77, H 9.91, N 7.59.

4-[Isopropyl(dimethyl)ammonio]butanoate (46). Compound **46** was obtained from *N*-isopropyl-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**36**) as hygroscopic crystals by a similar protocol as **14**; yield 56%, mp 53−54 °C. ^{1}H NMR (D_2O, DSS) δ 1.39 (d, J = 6.6 Hz, 6H), 2.01 (m, 2H), 2.26 (t, J = 7.1 Hz, 2H), 3.00 (s, 6H), 3.28 (m, 2H), 3.74 (septet, J = 6.6 Hz, 1H). ^{13}C NMR (D_2O) δ 15.4, 18.7, 33.5, 47.2 [t, $^1J_{(N,C)}$ = 3.9 Hz], 62.0 [t, $^1J_{(N,C)}$ = 2.6 Hz], 64.8, 180.7. LC−MS (ESI) m/z 174 [M + H]$^+$. Anal. Calcd for $C_9H_{19}NO_2\cdot1.84H_2O$: C 52.37, H 11.08, N 6.79. Found: C 52.38, H 11.46, N 6.61.

4-[Dimethyl(prop-2-yn-1-yl)ammonio]butanoate (47). Compound **47** was obtained from 4-methoxy-*N*,*N*-dimethyl-4-oxo-*N*-(prop-2-yn-1-yl)butan-1-aminium bromide (**37**) as hygroscopic crystals by a similar protocol as **14**; yield 87%. ^{1}H NMR (D_2O, DSS) δ 2.03 (m, 2H), 2.28 (t, J = 7.1 Hz, 2H), 3.19 (s, 6H), 3.26 (t, J = 2.4 Hz, 1H), 3.44 (m, 2H), 4.26 (d, J = 2.4 Hz, 2H). ^{13}C NMR (D_2O) δ 19.1, 33.3, 50.3, 54.1, 63.6, 70.3, 83.0, 180.5. IR (film) 2967, 2125, 1576, 1481, 1398, 1313, 1260, 1144 cm^{-1}. HRMS m/z calcd for $C_9H_{16}NO_2$ [M + H]$^+$, 170.1181; found, 170.1181. Anal. Calcd for $C_9H_{15}NO_2\cdot0.39H_2O$: C 61.33, H 9.02, N 7.95. Found: C 61.35, H 9.95, N 7.83.

4-[(Chloromethyl)dimethylammonio]butanoate (48).[27] Compound **48** was obtained from *N*-(chloromethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium chloride (**38**) as hygroscopic crystals by a similar protocol as **14**; yield 91%, mp 104−104.5 °C. ^{1}H NMR (D_2O, DSS) δ 2.04 (m, 2H), 2.29 (t, J = 7.1 Hz, 2H), 3.23 (s, 6H), 3.48 (m, 2H), 5.17 (s, 2H). ^{13}C NMR (D_2O) δ 18.8, 33.1, 49.2, 62.6, 68.6, 180.3. HRMS m/z calcd for $C_7H_{15}ClNO_2$ [M + H]$^+$, 180.0791; found, 180.0779. Anal. Calcd for $C_7H_{14}ClNO_2\cdot0.22H_2O$: C 45.79, H 7.93, N 7.63. Found: C 45.80, H 8.15, N 7.55.

4-[(Bromomethyl)dimethylammonio]butanoate (49). Compound **49** was obtained from *N*-(bromomethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium chloride (**39**) as hygroscopic crystals by a similar protocol as **14**; yield 74%, mp 127−129 °C. ^{1}H NMR (D_2O, DSS) δ 2.05 (m, 2H), 2.33 (t, J = 7.1 Hz, 2H), 3.26 (s, 6H), 3.51 (m, 2H), 5.18 (s, 2H). ^{13}C NMR (D_2O) δ 19.0, 33.2, 50.3, 57.0, 63.6, 180.4. HRMS m/z calcd for $C_7H_{15}BrNO_2$ [M + H]$^+$, 224.0286; found, 224.0304. Anal. Calcd for $C_7H_{14}BrNO_2\cdot1.31H_2O$: C 33.94, H 6.76, N 5.65. Found: C 33.94, H 6.22, N 5.59.

4-[(Iodomethyl)dimethylammonio]butanoate (50). Compound **50** was obtained from *N*-(iodomethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium chloride (**40**) as hygroscopic crystals by a similar protocol as **14**; yield 71%, mp 136−138 °C. ^{1}H NMR (D_2O, DSS) δ 2.02 (m, 2H), 2.30 (t, J = 7.1 Hz, 2H), 3.26 (s, 6H), 3.49 (m, 2H), 5.20 (s, 2H). ^{13}C NMR (D_2O) δ 19.3, 30.8, 33.2, 51.6, 64.8, 180.4. HRMS m/z calcd for $C_7H_{15}INO_2$ [M + H]$^+$, 272.0148; found, 272.0145. Anal. Calcd for $C_7H_{14}INO_2\cdot H_2O$: C 29.08, H 5.58, N 4.84. Found: C 29.01, H 5.36, N 4.74.

4-[(Methoxymethyl)dimethylammonio]butanoate (51). Compound **51** was obtained from 4-methoxy-*N*-(methoxymethyl)-*N*,*N*-dimethyl-4-oxobutan-1-aminium chloride (**41**) as hygroscopic crystals by a similar protocol as **14**; yield 67%, mp 134−136 °C. ^{1}H NMR (D_2O, DSS) δ 1.99 (m, 2H), 2.26 (t, J = 7.1 Hz, 2H), 3.04 (s, 6H), 3.29 (m, 2H), 3.69 (s, 3H), 4.61 (s, 2H). ^{13}C NMR (D_2O) δ 18.7, 33.5, 47.0 [t, $^1J_{(N,C)}$ = 3.8 Hz], 60.6 [t, $^1J_{(N,C)}$ = 2.4 Hz], 60.7, 91.8 [t, $^1J_{(N,C)}$ = 2.9 Hz], 180.6. HRMS m/z calcd for $C_8H_{18}NO_3$ [M + H]$^+$, 176.1287; found, 176.1274. Anal. Calcd for $C_8H_{17}NO_3\cdot0.59H_2O$: C 51.70, H 9.86, N 7.54. Found: C 51.72, H 10.61, N 7.34.

4-[Dimethyl(vinyl)ammonio]butanoate (52). Compound **52** was obtained from *N*-(2-chloroethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**42**) as hygroscopic crystals by a similar protocol as **14**; yield 62%. The compound was unstable on prolonged storage even in a freezer. ^{1}H NMR (D_2O, DSS) δ 1.95 (m, 2H), 2.26 (t, J = 7.2 Hz, 2H), 3.27 (s, 6H), 3.48 (m, 2H), 5.63 (m, 1H), 5.74 (m, 1H), 6.36 (m, 1H). ^{13}C NMR (D_2O) δ 19.5, 33.3, 51.3 [t, $^1J_{(N,C)}$ = 2.6 Hz], 66.2, 113.2, 140.3 [t, $^1J_{(N,C)}$ = 4.1 Hz], 180.6. HRMS m/z calcd for $C_8H_{16}NO_2$ [M + H]$^+$, 158.1181; found, 158.1171. Anal. Calcd for $C_8H_{15}NO_2\cdot0.65H_2O$: C 56.88, H 9.73, N 8.29. Found: C 56.87, H 11.20, N 8.16.

3-Carboxy-*N*-(2-chloroethyl)-*N*,*N*-dimethylpropan-1-aminium Chloride (53).[27] *N*-(2-Chloroethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**42**) (7.477 g, 25.9 mmol) was dissolved in water (10 mL), passed through Amberlite IRA-410 (Cl) ion-exchange resin column (100 mL) slowly (ca. 0.5 mL/min), and eluted with water (control with 2% $AgNO_3$ solution). The eluate was evaporated, and the residue (~6 g) was dissolved in 1 N HCl (50 mL) and stirred for 15 h at 70 °C. The reaction mixture was evaporated and the residue was dried in vacuo over P_2O_5 to give 4.755 g (79%) of **53** as a yellowish solid. The purity of **53** can be increased by crystallization from acetonitrile. Thus, 2 g of the obtained material was crystallized from acetonitrile (120 mL) to afford 1.32 g of white crystalline **53** with mp 130−132 °C. ^{1}H NMR (D_2O, DSS) δ 2.11 (m, 2H), 2.52 (t, J = 7.0 Hz, 2H), 3.20 (s, 6H), 3.46 (m, 2H), 3.80 (t, J = 6.7 Hz, 2H), 4.03 (t, J = 6.7 Hz, 2H). ^{13}C NMR (D_2O) δ 17.5, 29.8, 35.2, 51.1 [t, $^1J_{(N,C)}$ = 3.4 Hz], 63.7 [t, $^1J_{(N,C)}$ ~ 2.5 Hz], 63.8 [t, $^1J_{(N,C)}$ ~ 2.5 Hz], 176.2. HRMS m/z calcd for $C_8H_{17}ClNO_2$ $[M - Cl^-]^+$, 194.0948; found, 194.0951. Anal. Calcd for $C_8H_{17}Cl_2NO_2 \cdot 0.49H_2O$ (3.7%): C 40.21, H 7.58, N 5.86. Found: C 40.20, H 7.63, N 5.66. Cl^- counterion titration: calcd 15.40%, found 15.23%.

***N*-(2-Bromoethyl)-3-carboxy-*N*,*N*-dimethylpropan-1-aminium Chloride (54).** A solution of *N*-(2-bromoethyl)-4-methoxy-*N*,*N*-dimethyl-4-oxobutan-1-aminium bromide (**43**) (1.1029 g, 3.31 mmol) in 2 N HCl (20 mL) was stirred at 85 °C for 5 h. The reaction mixture was evaporated and the residue was dissolved in water (15 mL), passed through Amberlite IRA-410 (Cl) ion-exchange resin column (50 mL) slowly (ca. 0.5 mL/min), and eluted with water (control with 2% $AgNO_3$ solution). The eluate was extracted with diethyl ether (3 × 25 mL) and the aqueous phase was evaporated. The residue was dissolved in ethanol (15 mL), precipitated by addition of diethyl ether (20 mL), filtered, and dried in vacuo over P_2O_5 to afford 0.660 g (72%) of **54** as white crystals, mp 98−102 °C. ^{1}H NMR (D_2O) δ 1.95 (m, 2H), 2.37 (t, J = 7.0 Hz, 2H), 3.02 (s, 6H), 3.27 (m, 2H), 3.60−3.72 (m, 4H). ^{13}C NMR (D_2O) δ 17.4, 20.4, 29.8, 50.7 [t, $^1J_{(N,C)}$ = 3.6 Hz], 63.3 [t, $^1J_{(N,C)}$ ~ 2.5 Hz], 63.6 [t, $^1J_{(N,C)}$ ~ 2.5 Hz], 176.2. HRMS m/z calcd for $C_8H_{17}BrNO_2$ $[M - Cl^-]^+$, 238.0443; found, 238.0456. Anal. Calcd for $C_8H_{17}BrClNO_2 \cdot 0.05H_2O \cdot 0.25EtOH$: C 35.57, H 6.53, N 4.88. Found: C 35.57, H 6.54, N 4.80. Cl^- counterion titration: calcd 12.91%, found 12.52%.

3-(Dimethylamino)cyclohexanecarboxylic Acid Hydrochloride (56). A mixture of 3-aminocyclohexanecarboxylic acid (in the form of racemic cis and trans isomer mixture) (**55**) (2.58 g, 17.9 mmol), 37% formaldehyde (7.2 mL, 92 mmol), and formic acid (11.0 mL, 191 mmol) was refluxed for 25 h and then cooled, and concentrated HCl (5 mL, 58 mmol) was added. The reaction mixture was evaporated and the residue (4.84 g) was dissolved in 2-propanol (10 mL) and sedimented by adding diethyl ether (35 mL). The mixture was allowed to stand for 1 h in a freezer and then the precipitate was filtered, washed successively with diethyl ether and petroleum ether, and dried in vacuo over P_2O_5 to furnish 3.5 g (93%) of known[36] **56**. ^{1}H NMR (D_2O, DSS) δ 1.38 (m, 1H), 1.50 (m, 2H), 1.65 (q, J = 12.0 Hz, 1H), 2.09 (m, 3H), 2.36 (m, 1H), 2.59 (tt, J = 3.4, 12.2 Hz, 1H), 2.90 (s, 6H), 3.36 (tt, J = 3.6, 12.0 Hz, 1H). LC−MS (ESI) m/z 172 $[M + H]^+$.

Methyl 3-(Dimethylamino)cyclohexanecarboxylate (57). To cold methanol (80 mL) slowly was added acetylchloride (1.9 mL, 25.0 mmol), followed by 3-(dimethylamino)cyclohexanecarboxylic acid hydrochloride (**56**) (2.599 g, 12.5 mmol), and the resulting solution was stirred for 24 h at 42 °C. The mixture was evaporated, dichloromethane (90 mL) and powdered K_2CO_3 (1.94 g, 14.1 mmol) were added to thr residue, and the mixture was stirred for 7 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated at 38 °C/17 mmHg to afford 2.12 g (76%) of known[36] **57**. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.02−1.32 (m, 4H), 1.68−1.83 (m, 3H), 1.95 (m, 1H), 2.15 (s, 6H), 2.22 (tt, J = 3.4, 11.6 Hz, 1H), 2.31 (tt, J = 3.5, 12.0 Hz, 1H), 3.58 (s, 3H).

3-(Methoxycarbonyl)-*N*,*N*,*N*-trimethylcyclohexanaminium Iodide (58). To a solution of methyl 3-(dimethylamino)-cyclohexanecarboxylate (**57**) (2.122 g, 11.36 mmol) in methanol (25 mL) was added iodomethane (2.1 mL, 34.1 mmol), and the mixture was stirred for 24 h at room temperature. Then the reaction mixture was supplemented with another portion of iodomethane (1.0 mL, 16.1 mmol) and stirring was continued for 24 h. The reaction mixture was evaporated, diethyl ether (60 mL) was added to the residue, and the mixture was placed into an ultrasound bath for 2 h. The crystals were filtered, washed with diethyl ether, and dried to afford 3.414 g (91%) of **58**. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.19−1.48 (m, 3H), 1.54 (q, J = 12.1 Hz, 1H), 1.89 (m, 2H), 2.13 (m, 1H), 2.35 (m, 1H), 2.46 (tt, J = 3.4, 12.0 Hz, 1H), 3.02 (s, 9H), 3.41 (tt, J = 3.1, 12.0 Hz, 1H), 3.63 (s, 3H). ^{13}C NMR (D_2O) δ 23.3, 24.9, 27.0, 27.6, 41.8, 50.8 [t, $^1J_{(N,C)}$ = 3.9 Hz], 52.4, 72.9, 177.0. LC−MS (ESI) m/z 200 $[M - I^-]^+$.

3-(Trimethylammonio)cyclohexanecarboxylate (59). Compound **59** was obtained from 3-(methoxycarbonyl)-*N*,*N*,*N*-trimethylcyclohexanaminium iodide (**58**) as hygroscopic crystals by a similar protocol as **14**; yield 91%. ^{1}H NMR (D_2O, DSS) δ 1.26 (dq, J = 3.6, 12.8 Hz, 1H), 1.42 (tq, J = 3.1, 12.8 Hz, 1H), 1.49 (m, 1H), 1.56 (q, J = 12.0 Hz, 1H), 1.92 (m, 1H), 2.04 (m, 1H), 2.24 (m, 1H), 2.29 (tt, J = 3.2, 12.1 Hz, 1H), 2.4 (m, 1H), 3.08 (s, 9H), 3.38 (tt, J = 3.2, 11.9 Hz, 1H). ^{13}C NMR (D_2O) δ 23.8, 25.2, 28.3, 29.0, 45.5, 50.6 [t, $^1J_{(N,C)}$ = 4.0 Hz], 73.6 [unresolved t, $^1J_{(N,C)}$ = 2.6 Hz], 183.1. HRMS m/z calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$, 186.1494; found, 186.1520. Anal. Calcd for $C_{10}H_{19}NO_2 \cdot 1.9\ H_2O$: C 54.72, H 10.47, N 6.38. Found: C 54.73, H 10.69, N 6.36.

4-Iodopentanoic Acid (61). To a solution of NaI (54.0 g, 360.2 mmol) in acetonitrile (250 mL) was added (±)-γ-valerolactone (**60**) (25 mL, 269 mmol), followed by chlorotrimethylsilane (40 mL, 363 mmol), and the obtained mixture was stirred for 3 h at 80 °C. The reaction mixture was cooled, poured into ice water (600 mL), and stirred for 20 min. The mixture was extracted with diethyl ether (4 × 200 mL), washed successively with water (2 × 200 mL), 5% $Na_2S_2O_3$ solution (100 mL), and saturated NaCl solution (2 × 100 mL), and dried (Na_2SO_4). The solvents were evaporated at 20 °C/100 mmHg to give 40.655 g (66%) of known[37] crude **61** as an oil, extremely unstable on storage. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.94 (d, J = 6.9 Hz, 3H), 1.92−2.11 (m, 2H), 2.45−2.65 (m, 2H), 4.20 (m, 1H), 11.00 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.0, 28.8, 34.2, 37.2, 178.4. Under GC−MS assay conditions, **61** cyclized into starting γ-valerolactone (**60**): GC−MS (EI, 70 eV) m/z (I_{rel}, %) 56.1 $[M - HI - CO_2]^+$ (100), 85.0 $[M - HI - CH_3]^+$ (55), 100.0 $[M - HI]^+$ (7). Under LC−MS assay conditions, only the presence of I^- was observed, indicating facile formation of **60**: LC−MS (ESI) m/z 126.9 $[I]^-$, 172.9 $[I + HCOOH]^-$.

Methyl 4-Iodopentanoate (62). To cold methanol (250 mL) slowly was added acetylchloride (20 mL, 263 mmol), followed by 4-iodopentanoic acid (**61**) (40.655 g, 178 mmol), and the obtained solution was stirred for 1 h at ice-bath temperature and overnight at ambient temperature. The reaction mixture was poured into ice water (500 mL), extracted with diethyl ether (2 × 200 mL), washed successively with saturated NaCl solution (100 mL), saturated $NaHCO_3$ solution (100 mL), 5% $Na_2S_2O_3$ solution (100 mL), and saturated NaCl solution (2 x100 mL), and dried (Na_2SO_4). The extract was evaporated at 30 °C/70 mmHg and the residue (31.045 g) was chromatographed on silica gel (250 g) with chloroform−petroleum ether (gradient from 9:1 to 1:1) as eluent to afford 30.387 g (70%) of **62**. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.94 (d, J = 6.9 Hz, 3H), 1.91−2.12 (m, 2H), 2.39−2.60 (m, 2H), 3.68 (s, 3H), 4.20 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 28.5, 28.9, 34.3, 37.6, 51.7, 173.0. GC−MS (EI, 70 eV) m/z (I_{rel}, %) 115.1 $[M - I]^+$ (100), 168.9 $[M - CH_2COOCH_3]^+$ (5), 211.0 $[M - CH_3O]^+$ (16).

4-(Trimethylammonio)pentanoate (64).[38] Methyl 4-iodopentanoate (**62**) (8.713 g, 36 mmol) was dissolved in 33% trimethylamine solution in ethanol (20 mL, ca. 51 mmol), and the resulting mixture was stirred in a closed vessel at 50 °C for 48 h. The reaction mixture was cooled to room temperature, and the precipitate was filtered and washed with diethyl ether. The filtrates were combined, the solvents were evaporated, and the solid residue was mixed with diethyl ether (50 mL) and filtered. The combined portions of the solid material (7.064 g), consisting of 5-methoxy-*N*,*N*,*N*-trimethyl-5-oxopentan-2-aminium iodide (**63**) and trimethylammonium iodide, were dissolved in water (20 mL) and allowed to soak into Amberlite IRA-410 (OH) ion-exchange resin column (100 mL), and the column was stopped. After 0.5 h the column slowly (ca. 0.2 mL/min) was eluted with water. The eluate containing expected product (TLC control) was evaporated and the residue was dried in vacuo over P_2O_5 to afford **64** (1.819 g, 32%) as

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hygroscopic crystals (caution: evaporation of trimethylamine!), mp 197 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.23 (d, J = 6.5 Hz, 3H), 1.34 (tdd, J = 5.8, 10.6, 12.5 Hz, 1H), 1.76 (ddd, J = 6.1, 9.3, 15.3 Hz, 1H), 1.94 (ddd, J = 5.5, 6.3, 15.3 Hz, 1H), 2.14 (m, 1H), 2.98 (s, 9H), 3.42 (dqd, J = 2.3, 6.5, 10.6 Hz, 1H, partially overlapped with H_2O). ^{13}C NMR (D_2O) δ 12.6, 26.3, 34.1, 50.5 [t, $^1J_{(N,C)}$ = 4.0 Hz], 70.9 [t, $^1J_{(N,C)}$ = 2.0 Hz], 180.8. HRMS m/z calcd for $C_8H_{18}NO_2$ $[M + H]^+$, 160.1338; found, 160.1357. Anal. Calcd for $C_8H_{17}NO_2 \cdot 1.69\ H_2O$: C 50.66, H 10.83, N 7.38. Found: C 50.69, H 11.01, N 7.17.

3-Carboxy-2-chloro-*N*,*N*,*N*-trimethylpropan-1-aminium Chloride (65). Compound **65** was synthesized from DL-carnitine hydrochloride according to a literature procedure;[39] mp 159−162 °C (decomp). ^{1}H NMR (D_2O, DSS) δ 3.03 (dd, J = 6.8, 17.5 Hz, 1H), 3.05 (dd, J = 6.7, 17.5 Hz, 1H), 3.29 (s, 9H), 3.88 (dd, J = 1.6, 14.9 Hz, 1H), 3.94 (dd, J = 8.5, 14.9 Hz, 1H), 4.87 (m, 1H). ^{13}C NMR (D_2O) δ 41.5, 48.9, 53.9, 70.9, 173.0. HRMS m/z calcd for $C_7H_{15}ClNO_2$ $[M - Cl^-]^+$, 180.0791; found, 180.0793. Anal. Calcd for $C_7H_{15}Cl_2NO_2$: C 38.91, H 7.00, N 6.48. Found: C 38.99, H 6.96, N 6.36. Cl^- counterion titration: calcd 16.40%, found 16.62%.

(*E*)-4-Ethoxy-*N*,*N*,*N*-trimethyl-4-oxobut-2-en-1-aminium Bromide (67). To a solution of 20% trimethylamine in ethanol (5.6 mL, 16.9 mmol) at ice-bath temperature was added ethyl 4-bromocrotonate (**66**) (0.7 g, 3.6 mmol) dropwise. The mixture was stirred in a closed vessel at room temperature for 5 days and evaporated. The residue was washed in an ultrasound bath successively with diethyl ether (4 × 5 mL) and ethyl acetate (4 × 5 mL), sedimented from 2-propanol/diethyl ether (5 and 20 mL, accordingly), and triturated with ethyl acetate (10 mL). The precipitate was filtered and dried in vacuo over P_2O_5 to afford 0.7615 g (83%) of **67** as white hygroscopic crystals, mp 113−126 °C. ^{1}H NMR (D_2O, DSS) δ 1.32 (t, J = 7.1 Hz, 3H), 3.18 (s, 9H), 4.16 (d, J = 7.6 Hz, 1H), 4.29 (q, J = 7.1 Hz, 2H), 6.41 (d, J = 15.6 Hz, 1H), 7.03 (td, J = 7.6, 15.6 Hz, 1H). ^{13}C NMR (D_2O) δ 13.2, 53.0 [t, $^1J_{(N,C)}$ = 3.8 Hz], 62.2, 65.9 [t, $^1J_{(N,C)}$ = 3.2 Hz], 132.1, 132.6, 166.7. LC−MS (ESI) m/z 172 $[M - Br^-]^+$. Anal. Calcd for $C_9H_{18}BrNO_2 \cdot 0.66H_2O$: C 40.94, H 7.37, N 5.30. Found: C 40.90, H 7.14, N 5.74.

(*E*)-4-(Trimethylammonio)but-2-enoate (68).[16] Compound **68** was obtained from (*E*)-4-ethoxy-*N*,*N*,*N*-trimethyl-4-oxobut-2-en-1-aminium bromide (**67**) as hygroscopic crystals by a similar protocol as **14**; yield 72%, mp >100 °C (decomp). ^{1}H NMR (D_2O, DSS) δ 3.14 (s, 9H), 4.06 (d, J = 7.5 Hz, 2H), 6.33 (d, J = 15.4 Hz, 1H), 6.58 (td, J = 7.5, 15.4 Hz, 1H). ^{13}C NMR (D_2O) δ 52.6 [t, $^1J_{(N,C)}$ = 4.0 Hz], 66.4 [t, $^1J_{(N,C)}$ = 3.2 Hz], 126.8, 139.0, 172.9. HRMS m/z calcd for $C_7H_{14}NO_2$ $[M + H]^+$, 144.1025; found, 144.0998. Anal. Calcd for $C_7H_{13}NO_2 \cdot 0.42H_2O$: C 55.77, H 9.25, N 9.29. Found: C 55.75, H 9.54, N 9.33.

3-(Trimethylammonio)propane-1-sulfonate (70).[17] To a solution of sodium hydroxide (1.2 g, 30 mmol) in water (4 mL) was added trimethylamine hydrochloride (2.1 g, 22 mmol). The mixture was stirred for 30 min and extracted with benzene (6 × 5 mL). The extract was dried (Na_2SO_4) and filtered, and to the filtrate was added 1,3-propanesultone (**69**) (0.57 g, 4.7 mmol) solution in benzene (5 mL). The reaction mixture was stirred at room temperature for 3 days in a closed vessel, supplemented with ethyl acetate (25 mL), and the precipitate was filtered. The solid material was crystallized from methanol (15 mL) at freezer temperature (ca. −20 °C) and dried in vacuo over P_2O_5 to afford 0.2586 g (31%) of **70** as white crystals, mp >300 °C. ^{1}H NMR (D_2O, DSS) δ 2.26 (m, 2H), 3.00 (t, J = 7.2 Hz, 2H), 3.17 (s, 9H), 3.50 (m, 2H). ^{13}C NMR (D_2O) δ 18.4, 47.2 [t, $^2J_{(N,C)}$ = 1.6 Hz], 52.8 [t, $^1J_{(N,C)}$ = 4.0 Hz], 64.6 [t, $^1J_{(N,C)}$ = 3.1 Hz]. HRMS m/z calcd for $C_6H_{16}NO_3S$ $[M + H]^+$, 182.0851; found, 182.0876. Anal. Calcd for $C_6H_{15}NO_3S$: C 39.76, H 8.34, N 7.73. Found: C 39.85, H 8.57, N 7.62.

3-(Ethyldimethylammonio)propane-1-sulfonate (71).[17] To a solution of dimethylethylamine (0.73 g, 10 mmol) in 1,2-dichloroethane (5 mL) was added a solution of 1,3-propanesultone (**69**) (1.08 g, 8.8 mmol) solution in 1,2-dichloroethane (2 mL). The reaction mixture was stirred at room temperature for 2 days in a closed vessel, supplemented with ethyl acetate (15 mL), and the precipitate was filtered. The solid material (1.52 g) was crystallized from methanol/ethyl acetate (15 and 11 mL, correspondingly) in a refrigerator and dried in vacuo over P_2O_5 to afford 1.15 g (59%) of **71** as white crystals, mp >300 °C. ^{1}H NMR (D_2O, DSS) δ 1.37 (t, J = 7.0 Hz, 3H), 2.22 (quintet, J = 7.7 Hz, 2H), 3.00 (t, J = 7.1 Hz, 2H), 3.08 (s, 6H), 3.38−3.49 (m, 4H). ^{13}C NMR (D_2O) δ 7.3, 18.0, 47.2, 49.9 [t, $^1J_{(N,C)}$ = 4.0 Hz], 59.8 [t, $^1J_{(N,C)}$ = 2.6 Hz], 61.4 [t, $^1J_{(N,C)}$ = 3.0 Hz]. LC−MS (ESI) m/z 196 $[M + H]^+$. HRMS m/z calcd for $C_7H_{18}NO_3S$ $[M + H]^+$, 196.1007; found, 196.1026. Anal. Calcd for $C_7H_{17}NO_3S$: C 43.06, H 8.77, N 7.17. Found: C 43.25, H 9.15, N 7.10.

3-(Diethoxyphosphoryl)-*N*,*N*,*N*-trimethylpropan-1-aminium Bromide (73). To a solution of 20% trimethylamine in ethanol (17.0 mL, 51.2 mmol) was added diethyl (3-bromopropyl)phosphonate (**72**) (4.226 g, 16.3 mmol). The mixture was stirred in a closed vessel at room temperature for 5 days and evaporated. The residue was azeotropically dried with 2-propanol and crystallized from ethyl acetate (30 mL) by adding diethyl ether (10 mL). The crystals were dried in vacuo over P_2O_5 to give 5.0177 g (96%) of **73**, mp 102−108 °C. ^{1}H NMR (D_2O, DSS) δ 1.35 (t J = 7.2 Hz, 6H), 1.93−2.16 (m, 4H), 3.16 (s, 9H), 3.42 (m, 2H), 4.18 (m, 4H). ^{13}C NMR (D_2O) δ 15.5 [d, $^3J_{(P,C)}$ = 5.7 Hz], 16.0 [d, $^2J_{(P,C)}$ = 4.0 Hz], 20.7 [d, $^1J_{(P,C)}$ = 142.3 Hz], 52.8 [t, $^1J_{(N,C)}$ = 3.9 Hz], 63.5 [d, $^2J_{(P,C)}$ = 6.6 Hz], 65.8 [td, $^1J_{(N,C)}$ = 3.2 Hz, $^3J_{(P,C)}$ = 20.8 Hz]. LC−MS (ESI) m/z 238 $[M - Br^-]^+$. Anal. Calcd for $C_{10}H_{25}BrNO_3P \cdot 0.1H_2O$: C 37.54, H 7.94, N 4.38. Found: C 37.55, H 8.18, N 4.28.

***N*,*N*,*N*-Trimethyl-3-phosphonopropan-1-aminium Chloride (74).**[40] 3-(Diethoxyphosphoryl)-*N*,*N*,*N*-trimethylpropan-1-aminium bromide (**73**) (5.58 g, 17.5 mmol) was dissolved in water (10 mL), passed through Amberlyst A-21 (Cl) ion-exchange resin column (110 mL) slowly (ca. 0.5 mL/min), and eluted with water. The eluate was evaporated, the residue was azeotropically dried with 2-propanol followed by drying in vacuo over P_2O_5 to give 4.7 g (98%) of 3-(diethoxyphosphoryl)-*N*,*N*,*N*-trimethylpropan-1-aminium chloride as was suggested by elemental analysis. Anal. Calcd for $C_{10}H_{25}ClNO_3P \cdot 1.3H_2O$: C 40.42, H 9.36, N 4.71. Found: C 40.43, H 9.63, N 4.56.

Above-prepared 3-(diethoxyphosphoryl)-*N*,*N*,*N*-trimethylpropan-1-aminium chloride (4.7 g, 16.0 mmol) was refluxed with concentrated HCl (160 mL) for 24 h. The reaction mixture was evaporated and the residue was supplemented with water (25 mL), again evaporated, and azeotropically dried with 2-propanol. The residue (3.515 g) was crystallized from absolute ethanol (90 mL) in a freezer and dried in vacuo over P_2O_5 to afford 2.45 g (70%) of **74**. ^{1}H NMR (D_2O, DSS) δ 1.77 (td, J = 7.9, 18.1 Hz, 2H), 2.00−2.14 (m, 2H), 3.15 (s, 9H), 3.38−3.46 (m, 2H). ^{13}C NMR (D_2O) δ 16.6 [d, $^2J_{(P,C)}$ = 3.2 Hz], 23.1 [d, $^1J_{(P,C)}$ = 138.1 Hz], 52.8 [t, $^1J_{(N,C)}$ = 4.0 Hz], 66.2 [td, $^1J_{(N,C)}$ = 2.8 Hz, $^3J_{(P,C)}$ = 20.2 Hz]. LC−MS (ESI) m/z 182 $[M - Cl^-]^+$. HRMS m/z calcd for $C_6H_{17}NO_3P$ $[M - Cl^-]^+$, 182.0946; found, 182.0967. Anal. Calcd for $C_6H_{17}ClNO_3P$: C 33.11, H 7.87, N 6.44. Found: C 33.30, H 7.94, N 6.30. Cl^- counterion titration: calcd 16.29%, found 15.66%.

Ethyl (Diethoxymethyl)phosphinate (75). Compound **75** was obtained from hypophosphorous acid and triethyl orthoformate according to a literature procedure.[18]

Ethyl (3-Bromopropyl)(diethoxymethyl)phosphinate (76). To a suspension, prepared from 60% sodium hydride in mineral oil (0.43 g, 10.8 mmol) and tetrahydrofuran (3 mL), at ice-bath temperature slowly was added a solution of ethyl (diethoxymethyl)phosphinate (**75**) (1.96 g, 10.0 mmol) in tetrahydrofuran (3 mL). The mixture was stirred in the ice bath for 1 h and then slowly was transferred into a cold (10 °C) solution of 1,3-dibromopropane (5.47 g, 27.1 mmol) in tetrahydrofuran (5 mL). The resulting mixture was stirred at room temperature overnight, poured into ice water (25 mL), and extracted with ethyl acetate (3 × 25 mL). The extract was washed with saturated NaCl (25 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel with ethyl acetate as eluent to afford 0.7469 g (23%) of **76**. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.25 (t, J = 7.0 Hz, 3H), 1.26 (t, J = 7.0 Hz, 3H), 1.33 (t, J = 7.0 Hz, 3H), 1.84−2.02 (m, 2H), 2.09−2.30 (m, 2H), 3.47 (t, J = 6.5 Hz, 2H), 3.68 (qd, J = 7.0, 9.4 Hz, 1H), 3.71 (qd, J = 7.0, 9.4 Hz, 1H), 3.84 (qd, J = 7.0, 9.4 Hz, 1H), 3.85 (qd, J = 7.0, 9.4 Hz, 1H), 4.13 (qd, J = 7.0, 10.1 Hz, 1H), 4.22 (qd, J = 7.0, 10.1 Hz, 1H), 4.66 (d, J = 6.7 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 15.2, 16.7 [d, $^3J_{(P,C)}$ = 5.1 Hz], 24.2 [d, $^1J_{(P,C)}$ = 114.9 Hz], 24.6 [d, $^2J_{(P,C)}$ = 21.1 Hz], 34.0 [d, $^3J_{(P,C)}$ = 16.1 Hz], 61.6 [d, $^2J_{(P,C)}$ = 6.8 Hz], 65.6 [d, $^3J_{(P,C)}$ = 8.8 Hz], 101.0 [d, $^1J_{(P,C)}$ = 143.5 Hz]. LC−MS (ESI) m/z 317 $[M + H]^+$, 339 $[M + Na]^+$.

3-[(Diethoxymethyl)(ethoxy)phosphoryl]-*N*,*N*,*N*-trimethylpropan-1-aminium Bromide (77a). A mixture of ethyl (3-bromopropyl)(diethoxymethyl)phosphinate (**76**) (0.731 g, 2.3 mmol), 20% trimethylamine solution in ethanol (6 mL, 14 mmol), and acetonitrile (3 mL) was stirred at room temperature for 4 days. The solvents were evaporated and the residue was dried in vacuo over P_2O_5 to afford 0.9298 g (theoretical amount 0.867 g) of **77a**. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.23 (t, J = 7.0 Hz, 3H), 1.25 (t, J = 7.0 Hz, 3H), 1.34 (t, J = 7.0 Hz, 3H), 1.76−2.02 (m, 2H), 2.09−2.23 (m, 2H), 3.47 (s, 9H), 3.67 (qd, J = 7.0, 9.4 Hz, 1H), 3.71 (qd, J = 7.0, 9.4 Hz, 1H), 3.76 (m, 2H, overlapped with OCH_2CH$_3$ signals), 3.83 (qd, J = 7.0, 9.4 Hz, 1H), 3.85 (qd, J = 7.0, 9.4 Hz, 1H), 4.14 (qd, J = 7.0, 10.0 Hz, 1H), 4.20 (qd, J = 7.0, 10.0 Hz, 1H), 4.68 (d, J = 6.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 15.4, 15.5, 16.1 [d, $^3J_{(P,C)}$ = 3.8 Hz], 16.8 [d, $^2J_{(P,C)}$ = 5.2 Hz], 21.2 [d, $^1J_{(P,C)}$ = 89.0 Hz], 53.5 [t, $^1J_{(N,C)}$ = 3.0 Hz], 62.0 [d, $^2J_{(P,C)}$ = 7.0 Hz], 66.1 [d, $^3J_{(P,C)}$ = 9.0 Hz], 66.2 [d, $^3J_{(P,C)}$ = 9.0 Hz], 66.4 [td, $^1J_{(N,C)}$ = 2.2 Hz, $^3J_{(P,C)}$ = 10.4 Hz], 101.0 [d, $^1J_{(P,C)}$ = 146.5 Hz]. LC−MS (ESI) m/z 296 [M − Br$^-$]$^+$.

3-[(Diethoxymethyl)(ethoxy)phosphoryl]-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium Bromide (77b). A mixture of ethyl (3-bromopropyl)(diethoxymethyl)phosphinate (**76**) (1.3616 g, 4.29 mmol), dimethylethylamine (1.94 g, 26.6 mmol), and acetonitrile (10 mL) was stirred at room temperature for 9 days. The mixture was evaporated, and the residue was dissolved in water (25 mL) and washed with dichloromethane (3 × 15 mL). The aqueous phase was evaporated and the residue was dried in vacuo over P_2O_5 to afford 1.6354 g (97.6%) of **77b**. ^{1}H NMR (D_2O, DSS) δ 1.28 (t, J = 7.1 Hz, 3H), 1.28 (t, J = 7.1 Hz, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.33−1.41 (m, 3H), 1.93−2.17 (m, 4H), 3.09 (s, 6H), 3.39 (m, 2H), 3.43 (q, J = 7.3 Hz, 2H), 3.82 (qd, J = 7.1, 9.6 Hz, 1H), 3.83 (qd, J = 7.1, 9.6 Hz, 1H), 3.96 (qd, J = 7.1, 9.6 Hz, 1H), 3.97 (qd, J = 7.1, 9.6 Hz, 1H), 4.14−4.32 (m, 2H), 5.06 (d, J = 6.8 Hz, 1H). ^{13}C NMR (D_2O) δ 7.6, 14.6, 14.7, 15.8 [d, $^2J_{(P,C)}$ = 4.3 Hz], 16.1 [d, $^3J_{(P,C)}$ = 5.1 Hz], 20.6 [d, $^1J_{(P,C)}$ = 90.5 Hz], 50.2 [t, $^1J_{(N,C)}$ = 3.9 Hz], 60.0 [t, $^1J_{(N,C)}$ = 2.2 Hz], 63.0 [td, $^1J_{(N,C)}$ = 3.1 Hz, $^3J_{(P,C)}$ = 17.3 Hz], 63.9 [d, $^2J_{(P,C)}$ = 7.2 Hz], 67.7 [d, $^3J_{(P,C)}$ = 9.6 Hz], 67.8 [d, $^3J_{(P,C)}$ = 9.6 Hz], 100.5 [d, $^1J_{(P,C)}$ = 147.5 Hz]. LC−MS (ESI) m/z 310 [M − Br$^-$]$^+$.

[3-(Trimethylammonio)propyl]phosphinate (78). 3-[(Diethoxymethyl)(ethoxy)phosphoryl]-*N*,*N*,*N*-trimethylpropan-1-aminium bromide (**77a**) (0.8659 g, 2.3 mmol) was refluxed with 36% HCl (7 mL) for 22 h. The reaction mixture was evaporated and the residue was dissolved in water (5 mL), passed through Amberlite IRA-410 (OH) ion-exchange resin column, and slowly eluted with water. The eluate was treated with Dowex 50WX8 ion-exchange resin (0.3 g) for 1 min, and then the eluate was washed with diethyl ether (3 × 10 mL) and evaporated. The residue was azeotropically dried with 2-propanol, triturated in an ultrasound bath with acetone, filtered, and dried in vacuo over P_2O_5 to give 0.2351 g (62% on **76**) of **78**. ^{1}H NMR (D_2O, DSS) δ 1.57 (dtd, J = 1.6, 7.6, 15.1 Hz, 2H), 2.00 (m, 2H), 3.14 (s, 9H), 3.40 (m, 2H), 6.98 (td, J = 1.6, 511.1 Hz, 1H). ^{13}C NMR (D_2O, dioxan) δ 16.1, 28.2 [d, $^1J_{(P,C)}$ = 90.5 Hz], 53.5 [t, $^1J_{(C,N)}$ = 4.0 Hz], 67.3 [td, $^1J_{(C,N)}$ = 2.9 Hz, $^3J_{(P,C)}$ = 19.6 Hz]. HRMS m/z calcd for $C_6H_{17}NO_2P$ [M + H]$^+$, 166.0997; found, 166.0995. Anal. Calcd for $C_6H_{16}NO_2P\cdot0.45H_2O$: C 41.59, H 9.83, N 8.08. Found: C 41.59, H 10.36, N 7.98.

[3-(Ethyldimethylammonio)propyl]phosphinate (79). Compound **79** was obtained from [(diethoxymethyl)(ethoxy)phosphoryl]-*N*-ethyl-*N*,*N*-dimethylpropan-1-aminium bromide (**77b**) by a similar protocol as **78**; yield 47%. ^{1}H NMR (D_2O) δ 1.36 (tt, J = 1.9, 7.3 Hz, 3H), 1.57 (dtd, J = 1.6, 7.8, 15.1 Hz, 2H), 1.96 (m, 2H), 3.06 (s, 6H), 3.35 (m, 2H), 3.41 (q, J = 7.3 Hz, 2H), 6.98 (td, J = 1.6, 511.0 Hz, 1H). ^{13}C NMR (D_2O) δ 7.3, 15.1, 27.6 [d, $^1J_{(P,C)}$ = 89.7 Hz], 49.8 [t, $^1J_{(N,C)}$ = 3.9 Hz], 59.7 [t, $^1J_{(N,C)}$ = 2.6 Hz], 63.4 [td, $^1J_{(N,C)}$ = 2.6 Hz, $^3J_{(P,C)}$ = 19.2 Hz]. LC−MS (ESI) m/z 180 [M + H]$^+$. Anal. Calcd for $C_7H_{18}NO_2P\cdot1.03H_2O$: C 42.52, H 10.22, N 7.08. Found: C 42.52, H 10.50, N 6.88.

5-(3-Chloropropyl)-1*H*-tetrazole (81). To a solution of aluminum chloride (8.3 g, 62.0 mmol) in tetrahydrofuran (120 mL) was added sodium azide (12.87 g, 198.0 mmol). The reaction mixture was stirred for 15 min, 4-chlorobutyronitrile (**80**) (5.6 mL, 58.5 mmol) was added, and the mixture was refluxed for 30 h. To the mixture was added 15% HCl (ca. 90 mL) until the pH of the medium was 1. The hydrazoic acid was aspirated in vacuo and the product was extracted with ethyl acetate (4 × 150 mL). The extract was dried (Na_2SO_4) and evaporated. The residue (9.53 g) was supplemented with ice, and the pH of the medium was increased to 10 by addition of 2.5 N sodium hydroxide (ca. 20 mL). The mixture was washed with diethyl ether (4 × 50 mL) and the pH of the medium was brought back to 1−2 again with 15% HCl. The mixture was extracted with ethyl acetate (4 × 50 mL), and the extract was dried (Na_2SO_4) and evaporated. The residue was sedimented from diethyl ether/petroleum ether to afford 5.9 g of a solid material that was crystallized in a freezer from dichloromethane/petroleum ether (25 and 20 mL, accordingly) and dried in vacuo over P_2O_5 to afford 5.66 g (66%) of **81**, mp 48−50 °C. ^{1}H NMR ($CDCl_3$, HMDSO) δ 2.38 (tt, J = 6.1, 7.4 Hz, 2H), 3.32 (t, J = 7.4 Hz, 2H), 3.66 (t, J = 6.1 Hz, 2H), 12.74 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.8, 30.0, 43.5, 156.0. LC−MS (ESI) m/z 147 [M + H]$^+$. Anal. Calcd for $C_4H_7ClN_4\cdot0.13H_2O$: C 32.26, H 4.91, N 37.62. Found: C 32.23, H 4.85, N 37.64.

***N*,*N*,*N*-Trimethyl-3-(1*H*-tetrazol-5-yl)propan-1-aminium Chloride (82).** A mixture of 5-(3-chloropropyl)-1*H*-tetrazole (**81**) (2.066 g, 14.1 mmol) and 20% trimethylamine solution in ethanol (20 mL, 60.3 mmol) was stirred in a closed vessel at 55 °C for 78 h. The mixture was evaporated, and the residue was dissolved in water (15 mL) and washed successively with ethyl acetate (4 × 15 mL) and chloroform (4 × 15 mL). The aqueous solution was evaporated, and the residue was purified by reversed-phase column chromatography with water as eluent to give an oily substance that was sedimented from 2-propanol (10 mL) with ethyl acetate (35 mL). The precipitate was filtered and dried in vacuo over P_2O_5 to afford 1.485 g (51%) of **82** as white hygroscopic crystals, mp 95−130 °C. ^{1}H NMR (D_2O, DSS) δ 2.36 (m, 2H), 3.13 (t, J = 7.5 Hz, 2H), 3.17 (s, 9H), 3.45 (m, 2H). ^{13}C NMR (D_2O) δ19.6 [t, $^2J_{(N,C)}$ = 1.7 Hz], 20.2, 52.9 [t, $^1J_{(N,C)}$ = 4.0 Hz], 65.0 [t, $^1J_{(N,C)}$ = 3.2 Hz], 155.7. HRMS m/z calcd for $C_7H_{16}N_5$ [M − Cl$^-$]$^+$, 170.1406; found, 170.1343. Anal. Calcd for $C_7H_{16}ClN_5\cdot0.66H_2O$: C 38.64, H 8.02, N 32.19. Found: C 38.61, H 8.59, N 32.48. Cl$^-$ counterion titration: calcd 17.24%, found 16.29%.

4-Carboxy-*N*,*N*,*N*-trimethylbutan-1-aminium Bromide (84). A mixture of 5-bromopentanoic acid (**83**) (0.5559 g, 3.1 mmol), 20% trimethylamine solution in ethanol (4.5 mL, 13.6 mmol), and acetonitrile (3 mL) in a closed vessel was stirred at room temperature for 48 h. The mixture was evaporated and the residue was triturated with diethyl ether, filtered, and dried in vacuo over P_2O_5 to afford 0.5495 g (75%) of **84**, mp 152−156 °C. ^{1}H NMR (D_2O) δ 1.68 (quintet, J = 7.5 Hz, 2H), 1.85 (m, 2H), 2.49 (t, J = 7.3 Hz, 2H), 3.13 (s, 9H), 3.36 (m, 2H). ^{13}C NMR (D_2O) δ 20.9, 21.7, 32.9, 52.8 [t, $^1J_{(N,C)}$ = 4.0 Hz], 66.0 [t, $^1J_{(N,C)}$ = 2.8 Hz], 177.9. LC−MS (ESI) m/z 160.1 [M − Br]$^+$. Anal. Calcd for $C_8H_{18}BrNO_2\cdot0.19H_2O$: C 39.45, H 7.61, N 5.75. Found: C 39.45, H 7.96, N 5.60.

5-(Trimethylammonio)pentanoate (85).[19] Compound **85** was obtained from 4-carboxy-*N*,*N*,*N*-trimethylbutan-1-aminium bromide (**84**) as hygroscopic crystals by a similar protocol as **14**; yield 61%, mp 224−242 °C. ^{1}H NMR (D_2O) δ 1.43 (quintet, J = 7.5 Hz, 2H), 1.62 (m, 2H), 2.07 (t, J = 7.3 Hz, 2H), 2.92 (s, 9H), 3.15 (m, 2H). ^{13}C NMR (D_2O) δ 22.0, 22.4, 36.6, 52.7 [t, $^1J_{(N,C)}$ = 3.9 Hz], 66.2 [t, $^1J_{(N,C)}$ = 2.7 Hz], 182.5. HRMS m/z calcd for $C_8H_{18}NO_2$ [M + H]$^+$, 160.1338; found, 160.1349. Anal. Calcd for $C_8H_{17}NO_2\cdot1.55H_2O$: C 51.34, H 10.82, N 7.48. Found: C 51.35, H 11.40, N 7.37.

4-(Dimethylsulfonio)butanoate, Compound with Fumaric Acid, 1:1.14 (86). Fumaric acid (0.380 g, 3.28 mmol) and 4-(dimethylsulfonio)butanoate[41] (0.490 g, 3.28 mmol) were dissolved in methanol (3 mL) and the obtained solution was evaporated to dryness. The oily residue by trituration with acetone was converted into solid, which was filtered and dried in vacuo over P_2O_5 to afford white crystalline substance **86** (0.690 g), mp 131−133 °C. Further analysis of the obtained material by reversed-phase HPLC revealed that **86** consisted of 4-(dimethylsulfonio)butanoate and fumaric acid in a ratio of 1:1.14. ^{1}H NMR (D_2O, DSS) δ 2.12 (quintet, J = 7.4 Hz, 2H), 2.59 (t, J = 7.0 Hz, 2H), 2.92 (s, 6H), 3.36 (t, J = 7.8 Hz, 2H), 6.76 (s, 2.3H). ^{13}C NMR (D_2O) δ 18.8, 24.3, 32.0, 42.2, 134.4, 170.3, 176.7. HRMS m/z calcd for $C_6H_{13}O_2S$ [M − $C_4H_3O_4^-$], 149.0636; found, 149.0633. Anal. Calcd for $C_6H_{12}O_2S\cdot1.14C_4H_4O_4\cdot0.3H_2O$: C 44.36, H 6.05. Found: C 44.22, H 5.58.

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1-Ethyl-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium Bromide (88a). Compound **88a** was prepared as described in the literature.[42]

1-Allyl-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium Bromide (88b). A solution of methyl 3-(2,2-dimethylhydrazinyl)-propanoate **87**[20] (1.46 g, 10 mmol) and 3-bromopropene (1.21 g, 10 mmol) in ethanol (2 mL) was stirred at room temperature overnight. The solvent was evaporated, and the residue was washed with 2-propanol and diethyl ether and dried in vacuo over P_2O_5 to give 1.84 g (69%) of known[43] **88b** as an oil. ^{1}H NMR (DMSO-d_6, HMDSO) δ 2.50 (t, J = 6.6 Hz, 2H, overlapped with DMSO), 3.16 (td, J = 6.6, 7.5 Hz, 2H, overlapped with a signal of Me_2N), 3.18 (s, 6H), 3.63 (s, 3H), 4.15 (d, J = 7.1 Hz, 2H), 5.56−5.64 (m, 2H), 5.99 (tdd, J = 7.1, 9.8, 17.4 Hz, 1H), 6.37 (t, J = 7.5 Hz, 1H).

1-Isopropyl-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium Bromide (88c).[42] Compound **88c** was obtained from 3-(2,2-dimethylhydrazinyl)propanoate **87** and 2-bromopropane by a similar protocol as **88b**; oil, yield 62%. The reaction was performed in a sealed vessel at 70 °C for 12 h. ^{1}H NMR (D_2O, DSS) δ 1.42 (d, J = 6.5 Hz, 6H), 2.64 (t, J = 6.3 Hz, 2H), 3.21 (s, 6H), 3.30 (t, J = 6.3 Hz, 2H), 3.74 (s, 3H), 3.97 (septet, J = 6.5 Hz, 1H).

1-(2-Hydroxyethyl)-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium Bromide (88d).[43] Compound **88d** was obtained from 3-(2,2-dimethylhydrazinyl)propanoate **87** and 2-bromoethanol by a similar protocol as **88b**; oil, yield 83%. ^{1}H NMR (D_2O, DSS) δ 2.65 (t, J = 6.3 Hz, 2H), 3.31 (t, J = 6.3 Hz, 2H), 3.38 (s, 6H), 3.70−3.76 (m, 2H, overlapped with a signal of OCH_3), 3.74 (s, 3H), 4.08 (m, 2H).

1-(2-Chloroethyl)-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium Bromide (88e). Compound **88e** was obtained from 3-(2,2-dimethylhydrazinyl)propanoate **87** and 1-bromo-2-chloroethane by a similar protocol as **88b** as an oil after chromatographic purification on silica gel with chloroform−methanol (10:1), yield 67%. ^{1}H NMR (D_2O, DSS) δ 2.65 (t, J = 6.3 Hz, 2H), 3.31 (t, J = 6.3 Hz, 2H), 3.41 (s, 6H), 3.75 (s, 3H), 3.94−4.05 (m, 4H). ^{13}C NMR (D_2O) δ 31.3, 36.0, 38.2, 52.4, 52.9, 65.0, 174.1. LC−MS (ESI) m/z 209.1 $[M - Br^-]^+$.

2-(3-Methoxy-3-oxopropyl)-1,1-dimethyl-1-(prop-2-yn-1-yl)-hydrazin-1-ium Bromide (88f).[43] Compound **88f** was obtained from 3-(2,2-dimethylhydrazinyl)propanoate **87** and 3-bromoprop-1-yne by a similar protocol as **88b**; oil, yield 78%. ^{1}H NMR (D_2O, DSS) δ 2.67 (t, J = 6.3 Hz, 2H), 3.32 (t, J = 2.4 Hz, 1H), 3.34 (t, J = 6.3 Hz, 2H), 3.41 (s, 6H), 3.74 (s, 3H), 4.51 (d, J = 2.4 Hz, 2H).

Methyl 3-(1,2,2-Trimethylhydrazinyl)propanoate (89a). Compound **89a** was obtained from methyl acrylate and 1,1,2-trimethylhydrazine as described in the literature.[21]

Methyl 3-(1-Ethyl-2,2-dimethylhydrazinyl)propanoate (89b). To a solution of methyl 3-(2,2-dimethylhydrazinyl)propanoate (**87**) (1.46 g, 10 mmol) and acetaldehyde (0.66 g, 15 mmol) in methanol (20 mL) was added sodium cyanoborohydride (1.20 g, 20 mmol) portionwise at room temperature for 10 min. The mixture was stirred overnight and evaporated, and saturated NaCl (10 mL) was added to the residue. The mixture was extracted with ethyl acetate (4 × 20 mL), the extract was dried (Na_2SO_4) and evaporated, and the residue was distilled at 70−75 °C/15 mmHg to afford 1.18 g (68%) of **89b**. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.03 (t, J = 7.1 Hz, 6H), 2.26 (s, 6H), 2.45 (q, J = 7.1 Hz, 2H), 2.48 (t, J = 6.6 Hz, 2H), 2.71 (t, J = 6.6 Hz, 2H), 3.65 (s, 3H). GC−MS (EI, 70 eV) m/z (I_{rel}, %) 87.1 $[C_4H_{11}N_2]^+$ (100), 145.1 $[M - C_2H_5]^+$ (9), 159.1 $[M - CH_3]^+$ (15), 174.1 M^+ (51).

2-(3-Methoxy-3-oxopropyl)-1,1,1,2-tetramethylhydrazin-1-ium Iodide (90a). Compound **90a** was obtained from methyl 3-(1,2,2-trimethylhydrazinyl)propanoate (**89a**) and iodomethane by a similar protocol as **88b** as an oil. The crude product was used in the next step without additional purification. ^{1}H NMR (D_2O, DSS) δ 2.72 (t, J = 6.4 Hz, 2H), 2.76 (s, 3H), 3.28 (t, J = 6.4 Hz, 2H), 3.33 (s, 9H), 3.75 (s, 3H). ^{13}C NMR (D_2O) δ 32.7, 36.0, 47.7, 51.4, 52.4, 174.0. LC−MS (ESI) m/z 175.1 $[M - I^-]^+$.

1-Ethyl-2-(3-methoxy-3-oxopropyl)-1,1,2-trimethylhydrazin-1-ium Iodide (90b). Compound **90b** was obtained from methyl 3-(1,2,2-trimethylhydrazinyl)propanoate (**89a**) and iodoethane by a similar protocol as **88b** (the reaction was performed in a sealed vessel at 70 °C for 6 h) as an oil. The crude product was used in the next step without additional purification. ^{1}H NMR (D_2O, DSS) δ 1.33 (t, J = 7.1 Hz, 3H), 2.71 (s, 3H), 2.72 (t, J = 6.5 Hz, 2H), 3.23 (s, 6H), 3.25 (t, J = 6.5 Hz, 2H), 3.65 (q, J = 7.1 Hz, 2H), 3.74 (s, 3H). ^{13}C NMR (D_2O) δ 7.3, 32.6, 35.2, 46.9, 47.8, 52.3, 60.5, 174.1. LC−MS (ESI) m/z 189.1 $[M - Br^-]^+$.

2-(3-Methoxy-3-oxopropyl)-1,1,2-trimethyl-1-propylhydrazin-1-ium Iodide (90c). Compound **90c** was obtained from methyl 3-(1,2,2-trimethylhydrazinyl)propanoate (**89a**) and 1-iodopropane by a similar protocol as **88b** (the reaction was performed in a sealed vessel at 90 °C for 6 h) as an oil. The crude product was used in the next step without additional purification. ^{1}H NMR (D_2O, DSS) δ 0.96 (t, J = 7.4 Hz, 3H), 1.76 (m, 2H), 2.70 (t, J = 6.4 Hz, 2H), 2.70 (s, 3H), 3.24 (s, 6H), 3.25 (t, J = 6.4 Hz, 2H), 3.50 (m, 2H), 3.73 (s, 3H). ^{13}C NMR (D_2O) δ 9.5, 15.5, 32.6, 35.3, 47.0, 48.4, 52.3, 65.9, 174.1. LC−MS (ESI) m/z 203.2 $[M - I^-]^+$.

2-Ethyl-2-(3-methoxy-3-oxopropyl)-1,1,1-trimethylhydrazin-1-ium Iodide (90d). Compound **90d** was obtained from methyl 3-(1-ethyl-2,2-dimethylhydrazinyl)propanoate (**89b**) and iodomethane by a similar protocol as **88b** as an oil. The crude product was used in the next step without additional purification. ^{1}H NMR (D_2O, DSS) δ 1.21 (t, J = 7.2 Hz, 3H), 2.74 (t, J = 6.8 Hz, 2H), 3.14 (q, J = 7.2 Hz, 2H), 3.35 (s, 9H), 3.36 (t, J = 6.8 Hz, 2H), 3.75 (s, 3H). ^{13}C NMR (D_2O) δ 14.7, 34.0, 46.3, 47.0, 52.3, 52.3, 174.0. LC−MS (ESI) m/z 189.2 $[M - I^-]^+$.

3-(1-Ethyl-1,1-dimethylhydrazin-1-ium-2-yl)propanoate (91).[42] A solution of 1-ethyl-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium bromide (**88a**) (1.28 g, 5.0 mmol) in 80% ethanol (3 mL) was passed through a column (300 × 15 mm) with Amberlite IRA-410 (OH) ion-exchange resin slowly (ca. 0.2 mL/min) and eluted with 80% ethanol. The eluate containing expected product (TLC control) was evaporated and the residue was mixed with acetone. The precipitate was filtered, washed with 2-propanol and diethyl ether, and dried in vacuo over P_2O_5 to afford 0.64 g (80%) of **91** as white crystals, mp 182−184 °C. ^{1}H NMR (D_2O, DSS) δ 1.37 (t, J = 7.2 Hz, 3H), 2.39 (t, J = 6.5 Hz, 2H), 3.17 (t, J = 6.5 Hz, 2H), 3.27 (s, 6H), 3.59 (q, J = 7.2 Hz, 2H). LC−MS (ESI) m/z 161 $[M + H]^+$. ^{13}C NMR (D_2O) δ 7.3, 34.3, 39.5, 51.6, 60.5, 179.4. Anal. Calcd for $C_7H_{16}N_2O_2 \cdot 1.55H_2O$: C 44.69, H 10.23, N 14.89. Found: C 44.70, H 10.31, N 15.00.

3-(1-Allyl-1,1-dimethylhydrazin-1-ium-2-yl)propanoate (92).[43] Compound **92** was obtained from 1-allyl-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium bromide (**88b**) by a similar protocol as **91**; yield 71%, mp 158−160 °C. ^{1}H NMR (D_2O, DSS) δ 2.39 (t, J = 6.5 Hz, 2H), 3.23 (t, J = 6.5 Hz, 2H), 3.27 (s, 6H), 4.15 (d, J = 7.3 Hz, 2H), 5.73 (d, J = 17.1 Hz, 1H), 5.76 (d, J = 10.3 Hz, 1H), 6.05 (tdd, J = 7.3, 10.3, 17.1 Hz, 1H). LC−MS (ESI) m/z 173 $[M + H]^+$. Anal. Calcd for $C_8H_{16}N_2O_2 \cdot 0.82H_2O$: C 51.38, H 9.51, N 14.98. Found: C 51.39, H 9.89, N 14.93.

3-(1-Isopropyl-1,1-dimethylhydrazin-1-ium-2-yl)propanoate (93).[42] Compound **93** was obtained from 1-isopropyl-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium bromide (**88c**) by a similar protocol as **91**; yield 67%, mp 172−174 °C. ^{1}H NMR (D_2O, DSS) δ 1.42 (d, J = 6.6 Hz, 6H), 2.39 (t, J = 6.5 Hz, 2H), 3.19 (t, J = 6.5 Hz, 2H), 3.20 (s, 6H), 3.97 (septet, J = 6.6 Hz, 1H). ^{13}C NMR (D_2O) δ 15.5, 34.4, 39.2, 48.4, 66.7, 179.5. LC−MS (ESI) m/z 175 $[M + H]^+$. Anal. Calcd for $C_8H_{18}N_2O_2 \cdot 0.65H_2O$: C 51.67, H 10.46, N 15.06. Found: C 51.38, H 11.22, N 15.19.

3-[1-(2-Hydroxyethyl)-1,1-dimethylhydrazin-1-ium-2-yl]-propanoate (94). Compound **94** was obtained from 1-(2-hydroxyethyl)-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium bromide (**88d**) by a similar protocol as **91**; yield 74%, mp 155−157 °C. ^{1}H NMR (D_2O, DSS) δ 2.39 (t, J = 6.5 Hz, 2H), 3.20 (t, J = 6.5 Hz, 2H), 3.37 (s, 6H), 3.68−3.75 (m, 2H), 4.05−4.12 (m, 2H). ^{13}C NMR (D_2O) δ 34.3, 39.6, 52.8, 55.2, 66.3, 179.4. Anal. Calcd for $C_7H_{16}N_2O_3 \cdot 0.37H_2O$: C 45.97, H 9.23, N 15.32. Found: C 45.99, H 9.23, N 15.22.

3-(1,1-Dimethyl-1-vinylhydrazin-1-ium-2-yl)propanoate (95). Compound **95** was obtained from 1-(2-chloroethyl)-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazin-1-ium bromide (**88e**) by a similar protocol as **91**; yield 78%, mp 127−129 °C. ^{1}H NMR (D_2O, DSS) δ 2.38 (t, J = 6.6 Hz, 2H), 3.12 (t, J = 6.6 Hz, 2H), 3.46 (s, 6H), 5.75 (dd, J = 4.2, 8.3 Hz, 1H), 5.86 (dd, J = 4.2, 15.2 Hz, 1H), 6.37 (dd, J = 8.3, 15.2 Hz, 1H). ^{13}C NMR (D_2O) δ 34.3, 40.5, 53.0, 114.6, 140.1, 179.3. LC−MS (ESI) m/z 159.1 $[M + H]^+$. Anal. Calcd for

$C_7H_{14}N_2O_2 0.32H_2O$: C 51.28, H 9.00, N 17.09. Found: C 51.27, H 8.71, N 17.06.

2-(2-Carboxyethyl)-1-(2-chloroethyl)-1,1-dimethylhydrazin-1-ium Bromide (96). 1-(2-Chloroethyl)-2-(3-methoxy-3-oxopropyl)-1,1-dimethylhydrazinium bromide (**88e**) (1.45 g, 5.0 mmol) was dissolved in a mixture of water (20 mL) and concentrated HCl (5 mL). The obtained solution was stirred at 70−75 °C for 2 h and evaporated to dryness, and the residue was mixed with acetonitrile. The precipitate was filtered and dried in vacuo to afford 0.67 g (68%) of **96**, mp 105−107 °C. ^{1}H NMR (D_2O, DSS) δ 2.63 (t, J = 6.2 Hz, 2H), 3.29 (t, J = 6.2 Hz, 2H), 3.41 (s, 6H), 3.94−4.00 (m, 2H), 4.00−4.06 (m, 2H). ^{13}C NMR (D_2O) δ 31.4, 36.0, 38.2, 52.9, 65.0, 175.4. LC−MS (ESI) m/z 195 $[M - Br^-]^+$. Anal. Calcd for $C_7H_{16}BrClN_2O_2 \cdot 0.2H_2O$: C 30.12, H 5.92, N 10.03. Found: C 29.80, H 5.63, N 9.83. Br^- counterion titration: calcd 28.99%, found 26.27%.

2-(2-Carboxyethyl)-1,1-dimethyl-1-(prop-2-yn-1-yl)-hydrazin-1-ium Bromide (97).[43] Compound **97** was obtained from 2-(3-methoxy-3-oxopropyl)-1,1-dimethyl-1-(prop-2-yn-1-yl)hydrazin-1-ium bromide (**88f**) by a similar protocol as **96**; yield 71%, mp 113−115 °C. ^{1}H NMR (DMSO-d_6, HMDSO) δ 2.42 (t, J = 6.8 Hz, 2H), 3.13 (q, J = 7.0 Hz, 2H), 3.29 (s, 6H), 4.02 (t, J = 2.4 Hz, 1H), 4.58 (d, J = 2.4 Hz, 2H), 6.53 (t, J = 7.4 Hz, 1H), 12.43 (br s, 1H). IR (film) 3182, 3042, 2974, 2128, 1730, 1475, 1405, 1186 cm^{-1}. ^{13}C NMR (D_2O) δ 31.4, 38.6, 52.2, 55.5, 70.4, 82.1, 175.4. LC−MS (ESI) m/z 171 $[M - Br^-]^+$. HRMS m/z calcd for $C_8H_{15}N_2O_2$ $[M - Br^-]^+$, 171.1134; found, 171.1148. Anal. Calcd for $C_8H_{15}BrN_2O_2$: 38.26, H 6.02, N 11.16. Found: C 37.92, H 5.92, N 11.26. Br^- counterion titration: calcd 31.82%, found 29.38%.

3-(1,1,1,2-Tetramethylhydrazin-1-ium-2-yl)propanoate (98). Compound **98** was obtained from 2-(3-methoxy-3-oxopropyl)-1,1,1,2-tetramethylhydrazin-1-ium iodide (**90a**) by a similar protocol as **91**; yield 68% (on **89a**), mp 159−162 °C. ^{1}H NMR (D_2O, DSS) δ 2.43 (t, J = 6.6 Hz, 2H), 2.75 (s, 3H), 3.18 (t, J = 6.6 Hz, 2H), 3.31 (s, 9H). ^{13}C NMR (D_2O) δ 35.9, 36.2, 49.2, 51.3, 179.3. LC−MS (ESI) m/z 161 $[M + H]^+$. Anal. Calcd for $C_7H_{16}N_2O_2 \cdot 2.35H_2O$: C 41.51, H 10.30, N 13.83. Found: C 41.52, H 10.68, N 13.62.

3-(1-Ethyl-1,1,2-trimethylhydrazin-1-ium-2-yl)propanoate (99). Compound **99** was obtained from 1-ethyl-2-(3-methoxy-3-oxopropyl)-1,1,2-trimethylhydrazin-1-ium iodide (**90b**) by a similar protocol as **91**; yield 41% (on **89a**), mp 145−147 °C. ^{1}H NMR (D_2O, DSS) δ 1.35 (t, J = 7.2 Hz, 3H), 2.43 (t, J = 6.6 Hz, 2H), 2.70 (s, 3H), 3.15 (t, J = 6.6 Hz, 2H), 3.21 (s, 6H), 3.62 (q, J = 7.2 Hz, 2H). ^{13}C NMR (D_2O) δ 7.3, 35.2, 36.1, 47.7, 48.5, 60.4, 179.5. LC−MS (ESI) m/z 175 $[M + H]^+$. Anal. Calcd for $C_8H_{18}N_2O_2 0.77H_2O$: C 51.08, H 10.47, N 14.89. Found: C 51.07, H 10.44, N 14.85.

3-(1,1,2-Trimethyl-1-propylhydrazin-1-ium-2-yl)propanoate (100). Compound **100** was obtained from 2-(3-methoxy-3-oxopropyl)-1,1,2-trimethyl-1-propylhydrazin-1-ium iodide (**90c**) by a similar protocol as **91**; yield 48% (on **89a**), mp 144−146 °C. ^{1}H NMR (D_2O, DSS) δ 0.96 (t, J = 7.4 Hz, 3H), 1.76−1.89 (m, 2H), 2.42 (t, J = 6.6 Hz, 2H), 2.70 (s, 3H), 3.16 (t, J = 6.6 Hz, 2H), 3.23 (s, 6H), 3.45−3.52 (m, 2H). ^{13}C NMR (D_2O) δ 9.5, 15.5, 35.3, 36.0, 48.3, 48.5, 65.8, 179.4. LC−MS (ESI) m/z 189 $[M + H]^+$. Anal. Calcd for $C_9H_{20}N_2O_2 0.7H_2O$: C 53.81, H 10.74, N 13.95. Found: C 53.80, H 10.86, N 13.90.

3-(2-Ethyl-1,1,1-trimethylhydrazin-1-ium-2-yl)propanoate (101). Compound **101** was obtained from 2-ethyl-2-(3-methoxy-3-oxopropyl)-1,1,1-trimethylhydrazin-1-ium iodide (**90d**) by a similar protocol as **91**; yield 58% (on **89b**), mp 154−156 °C. ^{1}H NMR (D_2O, DSS) δ 1.23 (t, J = 7.2 Hz, 3H), 2.47 (t, J = 7.0 Hz, 2H), 3.12 (q, J = 7.2 Hz, 2H), 3.26 (t, J = 7.0 Hz, 2H), 3.33 (s, 9H). ^{13}C NMR (D_2O) δ 14.7, 37.3, 46.1, 48.5, 52.3, 179.4. LC−MS (ESI) m/z 175 $[M + H]^+$. Anal. Calcd for $C_8H_{18}N_2O_2 1.5H_2O$: C 47.74, H 10.52, N 13.92. Found: C 47.73, H 10.62, N 14.07.

1,1-Diethyl-2-(3-methoxy-3-oxopropyl)-1-methylhydrazin-1-ium Iodide (103).[43] Compound **103** was obtained from known[22] methyl 3-(2,2-diethylhydrazinyl)propanoate (**102**) and iodomethane by a similar protocol as **88b**; yield 72%.^{1}H NMR (D_2O, DSS) δ 1.36 (t, J = 7.2 Hz, 6H), 2.67 (t, J = 6.3 Hz, 2H), 3.18 (s, 3H), 3.24 (t, J = 6.3 Hz, 2H), 3.57 (m, 4H), 3.76 (s, 3H).

2-(2-Carboxyethyl)-1,1-diethyl-1-methylhydrazin-1-ium Chloride (104).[43] 1,1-Diethyl-2-(3-methoxy-3-oxopropyl)-1-methylhydrazin-1-ium iodide (**103**), obtained by a similar protocol as **91**, was converted into a glass-like solid. The obtained solid was dissolved in 6 N HCl, the solution was evaporated, and the residue was mixed with acetone. The precipitate was filtered and dried in vacuo over P_2O_5 to afford **104**, yield 74%, mp 150−152 °C. ^{1}H NMR (D_2O, DSS) δ 1.35 (t, J = 7.2 Hz, 6H), 2.63 (t, J = 6.3 Hz, 2H), 3.17 (s, 3H), 3.20 (t, J = 6.3 Hz, 2H), 3.47−3.63 (m, 4H). LC−MS (ESI) m/z 175 $[M - Cl^-]^+$. ^{13}C NMR (D_2O) δ 6.9, 31.4, 37.5, 48.1, 58.0, 175.6. Anal. Calcd for $C_8H_{19}ClN_2O_2 \cdot 1.9H_2O$: C 40.03; H 7.60; N 5.84. Found: C 40.07, H 7.84, N 5.63. Cl^- counterion titration: calcd 16.83%, found 16.77%.

3-(1-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}-1,1-dimethylhydrazin-1-ium-2-yl)propanoate (105). A mixture of methyl 3-(2,2-dimethylhydrazinyl)propanoate (**87**) (0.73 g, 5.0 mmol) and 2-(Boc-amino)ethyl bromide (1.12 g, 5.0 mmol) was left to stand overnight at room temperature. The reaction mixture was dissolved in 80% ethanol (5 mL), passed through a column (300 × 15 mm) with Amberlite IRA-410 (OH) ion-exchange resin slowly (ca. 0.2 mL/min), and eluted with 80% ethanol. The eluate containing expected product (TLC control) was evaporated and dried in vacuo over P_2O_5 to afford 0.95 g (69%) of **105** as an oil. ^{1}H NMR (D_2O, DSS) δ 1.45 (s, 9H), 2.40 (t, J = 6.5 Hz, 2H), 3.19 (t, J = 6.5 Hz, 2H), 3.36 (s, 6H), 3.57−3.68 (m, 4H). ^{13}C NMR (D_2O) δ 27.5, 34.1, 34.2, 39.6, 52.6, 63.4, 81.5, 157.7, 179.2. LC−MS (ESI) m/z 276.2 $[M + H]^+$.

3-[1-(2-Aminoethyl)-1,1-dimethylhydrazin-1-ium-2-yl]-propanoate (106). 3-(1-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}-1,1-dimethylhydrazin-1-ium-2-yl)propanoate (**105**) (0.70 g, 2.5 mmol) was dissolved in 6 N HCl (15 mL) and stirred at room temperature for 3 h. The solvent was evaporated and the residue was dissolved in 80% ethanol (5 mL), passed through a column (300 × 15 mm) with Amberlite IRA-410 (OH) ion-exchange resin slowly (ca. 0.2 mL/min), and eluted with 80% ethanol. The eluate containing expected product (TLC control) was evaporated and the residue was mixed with acetone. The precipitate was filtered and dried in vacuo over P_2O_5 to afford 0.30 g (67%) of **106** as white crystals, mp 81−83 °C. ^{1}H NMR (D_2O, DSS) δ 2.39 (t, J = 6.5 Hz, 2H), 3.10−3.16 (m, 2H), 3.20 (t, J = 6.5 Hz, 2H), 3.33 (s, 6H), 3.55−3.61 (m, 2H). ^{13}C NMR (D_2O) δ 34.3, 34.3, 39.6, 52.4, 65.6, 179.4. LC−MS (ESI) m/z 176 $[M + H]^+$. Anal. Calcd for $C_7H_{17}N_3O_2 \cdot 1.21H_2O$: C 42.67, H 9.93, N 21.33. Found: C 42.67, H 10.14, N 21.07.

Isopropyl 2-(2,2-Dimethylhydrazinyl)ethanesulfonate (108). A mixture of 1,1-dimethylhydrazine (0.6 g, 10 mmol) and isopropyl ethenesulfonate[23] (**107**) (1.5 g, 10 mmol) was left to stand overnight at room temperature to give **108** as a viscous oil (2.1 g), which was used in the next step without additional purification. ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.44 (d, J = 6.6 Hz, 6H), 2.34 (s, 1H), 2.45 (s, 6H), 3.03 (t, J = 6.3 Hz, 2H), 3.20 (t, J = 6.3 Hz, 2H), 3.97 (septet, J = 6.6 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 23.1, 43.0, 47.5, 50.4, 52.2. GC−MS (EI, 70 eV), m/z (I_{rel}, %) 59.1 $[C_3H_7O]^+$ (100), 85.1 $[C_4H_9N_2]^+$ (34), 123.0 $[M - C_4H_{11}N_2]^+$ (4), 151.1 $[M - C_3H_7O]^+$ (1.5), 168.1 $[M - C_3H_6]^+$ (28), 210.1 M^+ (1.5).

2-[2-(Isopropoxysulfonyl)ethyl]-1,1,1-trimethylhydrazin-1-ium Iodide (109). Compound **109** was obtained from isopropyl 2-(2,2-dimethylhydrazinyl)ethanesulfonate (**108**) and iodomethane by a similar protocol as **88b** as an oil, yield 72% (on **107**). ^{1}H NMR (D_2O, DSS) δ 1.45 (d, J = 6.6 Hz, 6H), 3.13 (t, J = 6.7 Hz, 2H), 3.39 (s, 9H), 3.45 (t, J = 6.7 Hz, 2H), 3.82 (septet, J = 6.6 Hz, 1H). ^{13}C NMR (D_2O) δ 23.7, 38.9, 48.1, 52.7, 54.3. LC−MS (ESI) m/z 183.1 $[M - I^- - C_3H_7 + H]$, 225.1 $[M - I^-]^+$.

2-(1,1,1-Trimethylhydrazin-1-ium-2-yl)ethanesulfonate (110). Compound **110** was obtained from 2-[2-(isopropoxysulfonyl)-ethyl]-1,1,1-trimethylhydrazin-1-ium iodide (**109**) by a similar protocol as **91**; yield 80%, mp 266−268 °C. ^{1}H NMR (D_2O, DSS) δ 3.13 (t, J = 6.6 Hz, 2H), 3. 39 (s, 9H), 3.45 (t, J = 6.6 Hz, 2H). ^{13}C NMR (D_2O) δ 38.9, 48.0, 54.3. LC−MS (ESI) m/z 183 $[M + H]^+$. Anal. Calcd for $C_5H_{14}N_2O_3S \cdot 0.1H_2O$: C 32.63, H 7.78, N 15.22. Found: C 32.65, H 8.12, N 15.27.

Diethyl [2-(2,2-Dimethylhydrazinyl)ethyl]phosphonate (112). To a solution of diethyl (2-(2,2-dimethylhydrazono)ethyl)-phosphonate[24] (**111**) (1.11 g, 5.0 mmol) in methanol (20 mL)was

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added sodium cyanoborohydride (0.62 g, 10 mmol) portionwise at room temperature for 10 min. The reaction mixture was acidified to pH 3–4 by 1 N hydrogen chloride solution in methanol, the mixture was stirred overnight and evaporated and water (10 mL) was added to the residue. The mixture was extracted with ethyl acetate (2 × 20 mL), and the extract was dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel with chloroform/methanol (15:1) as eluent to afford **112** as an oil (0.919 g, 82%). ^{1}H NMR ($CDCl_3$, HMDSO) δ 1.36 (t, J = 7.1 Hz, 6H), 2.10 (td, J = 6.9, 18.0 Hz, 2H), 2.89 (s, 6H), 3.16 (br s, 1H), 3.27 (td, J = 6.9, 17.7 Hz, 2H), 4.07–4.19 (m, 4H).

2-[2-(Diethoxyphosphoryl)ethyl]1,1,1-trimethylhydrazin-1-ium Iodide (113). Compound **113** was obtained from diethyl [2-(2,2-dimethylhydrazinyl)ethyl]phosphonate (**112**) and iodomethane by a similar protocol as **88b** as an oil after chromatographic purification on silica gel (eluent was methanol/chloroform, 1:5); yield 67%. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.25 (t, J = 7.1 Hz, 6H), 1.88 (td, J = 7.8, 18.5 Hz, 2H), 3.08 (qd, J = 7.7, 10.5 Hz, 2H), 3.25 (s, 9H), 3.98–4.07 (m, 4H), 6.38 (t, J = 7.5 Hz, 1H).

1,1,1-Trimethyl-2-(2-phosphonoethyl)hydrazin-1-ium Iodide (114). Compound **114** was obtained from 2-[2-(diethoxyphosphoryl)-ethyl]-1,1,1-trimethylhydrazin-1-ium iodide (**113**) by refluxing the latter in 6 N HCl for 15 h by a similar protocol as **96**; yield 72%, oil. ^{1}H NMR (DMSO-d_6, HMDSO) δ 1.68 (td, J = 8.1, 18.2 Hz, 2H), 3.06 (q, J = 8.2 Hz, 2H), 3.27 (s, 9H), ~6.6 (br s, 1H). ^{13}C NMR (D_2O) δ 25.0 [d, $^2J_{(P,C)}$ = 135.6 Hz], 38.1, 54.3. LC–MS (ESI) m/z 183 $[M - I^-]^+$. Anal. Calcd for $C_5H_{16}IN_2O_3P{\cdot}0.3H_2O$: C 19.04, H 5.30, N 8.88. Found: C 19.38, H 5.57, N 8.55.

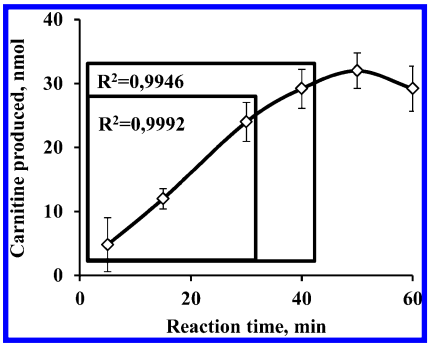

Figure 4. Human recombinant BBOX catalyzed carnitine production depending on reaction time. Results are mean ± SD.

BBOX Activity Determination. γ-Butyrobetaine hydroxylase (BBOX) activity was assayed by measuring the formation of carnitine from GBB and was carried out according to a slightly modified version of the method reported by Lindstedt and Lindstedt.[44] Human recombinant BBOX was used as an enzyme source. For the initial screening, the complete reaction mixture (final volume of 0.2 mL) contained 20 mM potassium phosphate, pH 7.0, 20 mM potassium chloride, 3 mM 2-oxoglutarate, 0.25 mM ferrous ammonium sulfate, 10 mM sodium ascorbate, 0.16 mg of catalase, 200 μM GBB, and 0.6 μg of BBOX. The mixture was preincubated for 15 min in the presence or absence (control) of the tested inhibitor (100 or 1000 μM). The reaction was initiated by adding GBB, and to ensure the linear rate range the mixture was incubated at 37 °C for 30 min (Figure 4). The reaction was stopped with 0.8 mL of ice-cold acetonitrile/methanol (1:3). Then, mixture was spun at 20000g for 10 min at 4 °C. The supernatant was decanted and used for carnitine measurements. The determination of L-carnitine was performed by ultraperformance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) in positive-ion electrospray mode, as previously described.[45] Compounds that decreased BBOX activity by at least 50% compared to the control were further tested to determine the IC_{50} value. The reaction mixture and treatment were as previously described, and 6–8 different concentrations (concentrations were chosen depending on the inhibitory effect observed during initial screening) of the tested compounds were used to obtain a dose–activity curve. GraphPad Prism 3.0 software was used to determine IC_{50} values of all tested compounds by logistic regression analysis. The IC_{50} values are presented as mean ± standard deviation of three independent experiments (for compounds with IC_{50} values less than 1000 μM). IC_{50} curves of 23 compounds with inhibition potency higher than that of reference compound (mildronate) are shown in Figure S1 in

Table 2. Data Processing, Refinement, and Validation Statistics

	BBOX structure in complex with inhibitor					
	14	**24**	**26**	**64**	**78**	**98**
space group	*H*32	*H*32	*H*32	*H*32	*H*32	*H*32
cell dimension $a = b$, Å	107.1	107.2	107.6	107.4	107.7	107.2
cell dimension c, Å	205.3	205.2	205.5	205.3	204.7	205.0
resolution, Å	29–1.70	40–2.40	27–1.88	27–2.05	38–2.18	29–2.15
highest-resolution shell, Å	1.70–1.79	2.40–2.53	1.88–1.98	2.05–2.16	2.18–2.30	2.15–2.27
no. reflcns	50 058	17 821	37 202	27 884	23 613	24 974
no. reflcns in test set	2540	906	1833	1421	1216	1267
completeness,[a] %	100 (100)	99 (100)	97 (82)	97 (99)	98 (99)	100 (100)
R_{merge}	0.08 (0.59)	0.10 (0.43)	0.08 (0.56)	0.09 (0.41)	0.13 (0.45)	0.09 (0.40)
$\langle I/\sigma I \rangle$	9.1 (2.1)	9.4 (2.6)	9.1 (1.9)	7.3 (2.3)	6.2 (2.2)	10.2 (2.9)
avg multiplicity	3.7 (3.7)	3.3 (3.3)	3.1 (2.8)	2.5 (2.4)	2.5 (2.2)	3.7 (3.7)
R-factor	0.16 (0.26)	0.16 (0.23)	0.16 (0.28)	0.15 (0.22)	0.15 (0.19)	0.16 (0.22)
R_{free}	0.20 (0.30)	0.23 (0.33)	0.20 (0.30)	0.21 (0.35)	0.19 (0.26)	0.22 (0.30)
avg B factor, Å^2	24.6	27.7	25.0	25.8	21.3	28.5
avg B factor for ligands and ions, Å^2	25.5	27.9	25.6	25.0	19.4	28.6
$\langle B \rangle$ from Wilson plot, Å^2	17.6	36.5	19.4	19.9	21.6	28.2
no. protein atoms	3173	3125	3150	3144	3130	3114
no. inhibitor atoms	10	12	12	11	10	11
no. solvent molecules	410	267	397	386	368	319
	RMS Deviations from Ideal Values					
bond lengths, Å	0.010	0.011	0.010	0.012	0.010	0.010
bond angles, deg	1.38	1.41	1.39	1.49	1.34	1.38
outliers in Ramachandran plot,[53] %	0.27	0.52	0.53	0.26	0.26	0.26

[a]Values in parentheses are for the high-resolution bin.

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Supporting Information. As a limitation of the reported IC_{50} values, it should be mentioned that no internal standards were included within the incubations to ensure that the compounds tested remain stable.

Crystallographic Studies. For crystallization of all inhibitor complexes, slightly modified earlier-described conditions[12] were used. Purified protein was concentrated to 7 mg/mL in a 10 kDa cutoff Amicon Ultra 0.5 mL concentrator (Millipore) and crystallized by the sitting drop vapor technique in MRC 96-well crystallization plates (Molecular Dimensions). Protein (1 μL) was mixed with 1 μL of bottom solution, supplemented with 8 mM *N*-oxalylglycine and 4 mM inhibitor. Bottom solution contained 0.2 M ammonium citrate, 20% poly-(ethylene glycol) (PEG) 3350, 3% hexanediamine, and 10 mM $ZnSO_4$, pH 7.0. The crystals were soaked for approximately 1 min in mother liquor supplemented with 30% glycerol and then flash-frozen in liquid nitrogen. Data were collected at beamlines I911-2 and I911-3, MAXlab, Lund, Sweden. Data were processed with MOSFLM[46] and scaled with SCALA.[47] All structures were solved by molecular replacement, with the BBOX structure in complex with GBB (PDB code 3O2G) as initial model. Parameter files for inhibitors and *N*-oxalylglycine were generated with SKETCHER, which is the CCP4[48] interface of LIBCHECK.[49] Initial phasing and refinement was done by REFMAC.[50] Model was built in COOT[51] followed by additional REFMAC runs. Data scaling, refinement, and validation statistics are shown in Table 2.

Isothermal Calorimetric Experiments. All experiments were performed with purified human BBOX in 50 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-ethanesulfonic acid (HEPES) buffer (pH = 7.4) with 100 mM KCl added for protein stabilization. All protein portions were dialyzed; the same dialysate was used to dissolve cofactors and ligands. To prevent instantaneous enzymatic reaction, the native cofactor and ion were replaced with isosteric ones: 2-ketoglutarate with *N*-oxalylglycine, and Fe(II) with Ni(II). Protein concentration was determined after addition of the cofactor by absorbance at 280 nm on a NanoDrop 2000c UV−vis spectrometer.

Enzyme concentration was varied from 26 to 75 μM (calculated relative to one subunit of the homodimer). Ligand concentrations were chosen according to the available IC_{50} values and then corrected after the initial ITC results. ITC was performed on a MicroCal iTC200 system at 25 °C. The first injection of 0.2 μL was followed by 19 injections of 2.0 μL at a steering rate of 500 rpm. All experiments were repeated at least three times.

Heats of dilution arising from ligand titration into the buffer were subtracted from the data before final curve-fitting. The first 0.2 μL injection has been excluded from each data set to remove the effect of titrant diffusion across the syringe tip during the equilibration process. Curve-fitting was performed by Origin v7.0 software. For all experiments $c < 5$ was found, and as a result, low-affinity experiments were used. Concentrations of ligands and protein were chosen to achieve final receptor occupancies of 90% for $c = 10$ and 80% for $c = 1$, 0.1, 0.01, and 0.001.[52] ITC titration curves are shown in Supporting Information (Figure S2).

■ ASSOCIATED CONTENT

Supporting Information

Two figures showing IC_{50} and ITC titration curves. This material is available free of charge via the Internet as http://pubs.acs.org.

Accession Codes

Atomic coordinates, along with experimental structure factors for crystal structures of the inhibitors complexed with BBOX, were deposited in the Protein Data Bank (ID code 4C5W for **14**, 4BGM for **24**, 4BG1 for **26**, 4BHF for **64**, 4BGK for **78**, and 4BHI for **98**).

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

GBB, γ-butyrobetaine; BBOX, γ-butyrobetaine hydroxylase; ITC, isothermal titration calorimetry; SAR, structure−activity relationship

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