Improved synthesis and in vitro/in vivo activities of natural product-inspired, artificial glutamate analogs

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1. Introduction

Glutamate receptors (GluRs) play a central role in rapid synaptic neurotransmission, and are involved in higher brain functions such as memory and learning. GluRs are also thought to be fully or partly involved in nociception, as well as in a number of brain disorders such as epilepsy, ischemia-induced excitotoxicity, Alzheimer's, Huntington's, and Parkinson's diseases, and schizophrenia. Thus, selective GluR ligands, or even biologically functional glutamate analogs, are of significant biomedical interest in neurobiology.

A variety of glutamate analogs have been isolated from natural resources and characterized pharmacologically, and a number of their analogs have been chemically synthesized. In the latter synthetic studies, three general approaches were used to establish structure-activity relationships: 1) structural modification of natural specimens by newly incorporating a substituent or a functional group, 2) de novo total synthesis of natural product and analogs, and 3) construction of combinatorial library of artificial compounds by diversity-oriented synthesis (DOS).

Within the context of the second approach, de novo synthesis, we have been studying the chemical synthesis and biological function of dysiherbaine and neodysiherbaine A, which are natural glutamate analogs isolated from Micronesian sponge, *L. chondrodes* (Figure 1). Dysiherbaines are now known to be subtype-selective agonists for kainate (KA) receptors and exhibit potent agonistic actions for two of those proteins, GluK1 (GluR5) and GluK2 (GluR6), with the highest affinity of all known ligands. By extending the natural product synthesis to analog synthesis, we discovered that MSVIII-19 (8,9-dideoxyneodysiherbaine A), which lacks functional groups at C8 and C9 positions, acts as a functional antagonist.

More recently, we began to pursue the third approach, DOS, and consequently discovered the artificial glutamate analog 1a, which elicits hypoactivity, rather than convulsions, in mice behavioral assays. Interestingly, 1a also markedly reduced both action potential firing frequency and spontaneous excitatory synaptic currents in current- and voltage-clamp electrophysiological analyses from cultured hippocampal neurons, although 1a did not displace radioactive ligands for the (S)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), kainate (KA), or N-methyl-D-aspartate (NMDA) receptors that are constituent members of the
ionotropic GluR superfamily. The mechanistic basis for these activities of 1a is under active investigation.

In these DOS studies, however, only four glutamate analogs were synthesized and biologically evaluated. Here, we report an improved synthetic route to a total of twelve artificial glutamate analogs. In vitro and in vivo biological evaluation of a subset of the compounds identified a new analog 7c as a ligand that potently altered neuronal excitation and synaptic activity.12

2. Results and discussions

2.1. Synthesis of twelve artificial glutamate analogs

Our first-generation synthesis11 of artificial glutamate analogs 1a, 1b, 5a, and 5b is shown in Scheme 1. The 7-oxanorbornenes 2a and 2b, readily available in 50% and 33% yields for two steps, respectively, were subjected to the challenging domino metathesis reaction using Hoveyda-Grubbs second-generation catalyst13 in the presence of electron-rich vinyl acetate as an unprecedented cross metathesis substrate, giving rise to heterotricycles 3a and 3b exclusively in 100% and 84% yields, respectively. Four-step transformation led 3a and 3b into diesters 4a and 4b in 76% and 58% yields. From the common intermediates 4a and 4b, two glutamate analogs bearing a saturated ring, 5a and 5b, were obtained in 25% and 43% yields for 4 steps, whereas two dihydroxylated analogs 1a and 1b were also furnished in 53% and 27% yields over 5 steps. However, the first-generation synthetic pathway (Scheme 1) included several problems to be solved. First, deoxygenation of the A ring pyrrolidone lactam with BH₃•Me₂S proceeded in only 40–62% yield. Second, since the borane reagent is highly reactive to olefins, the first-generation approach was not capable of synthesizing glutamate analogs with olefin functionality at the C ring. Third, the pathway was not applicable to a synthesis of glutamate analogs with an amino group at the C ring. This was because of steric interference caused by N-protecting groups to the reagents used in a series of reactions shown in Scheme 1. Forth, as a branching point,14 common intermediates 4a and 4b were at too early stages in the synthetic pathway (3 steps before 5a and 5b, and 5 steps before 1a and 1b). This was apparently inconvenient in terms of efficiency in diversity-oriented synthesis.

To solve these problems, we investigated a new efficient route in the present study that
resulted in a synthesis of total twelve glutamate analogs, including those bearing an amino group at the C ring, with good overall yield over a shorter series of reactions. The plan is shown in Scheme 2. Here, the advanced intermediates 6a–6d were placed two steps after the common intermediates 4a–4d, and diversified into three glutamate groups in 1–2 steps: a dihydroxylated group (1a–1d), a saturated group (5a–5d), and an unsaturated group (7a–7d).  

Scheme 3 depicts synthesis of the common intermediates 4c and 4d, bearing amino groups at the C rings, from known 3c and 3d, respectively, by four-step functional group transformations, which had been developed for the synthesis of 4a and 4b (see Scheme 1).  

First, N-Boc imides 8c and 8d were prepared by treatment with Boc₂O, triethylamine (TEA), and 4-(dimethylamino)pyridine (DMAP) in 90% and 83% yields, respectively. Alkaline hydrolysis (K₂CO₃, MeOH) was carefully performed on 8c and 8d, giving rise to ester aldehydes 9c and 9d in good yields (81% and 91%) without affecting β-alkoxyaldehyde moiety. The ester aldehydes in turn were oxidized by NaClO₂ followed by esterification (TMSCHN₂) to provide 4c and 4d in 93% and 85% yields, respectively.

The common intermediates 4a and 4b were also synthesized with a similar scheme as was reported recently in our first-generation synthesis.

An improved synthesis of the advanced intermediates 6a–6d from 4a–4d, employed also as common intermediates in our first-generation synthesis, is shown in Scheme 4. Here, the key transformation is deoxygenation of pyrrolidone lactam, which was successfully achieved by a two-step reaction including reduction of the imidate. Initially, the PMB group was removed oxidatively by ceric ammonium nitrate (CAN) at –10 °C to give 10a–10d in 71–80% yield. Upon treatment of 10a–10d with Meerwein reagent (Me₃O•BF₄) and K₂CO₃, corresponding imidates were readily generated. Without purification, the imidates were reduced with NaBH₃CN under acidic conditions (TFA, MeOH) to provide the corresponding pyrrolidines, which were subsequently protected by Boc group (Boc₂O, TEA) to furnish advanced intermediates 6a–6d in 59–86% yields over 3 steps. As compared to the first-generation synthesis (see Scheme 1), the yields and reproducibility of the deoxygenation steps were improved satisfactorily. For a convenient, one-step deprotection at the final stage of the synthesis, 2-nitrobenzenesulfonyl (Ns) group in 6c was replaced with Boc group by two-step transformation (PhSH, Cs₂CO₃; Boc₂O, TEA) to give 6c' in 86% yield.
It should be noted here that rhodium-catalyzed reduction reported by Kuwano et al.\textsuperscript{18} also worked for this transformation. However, the yield was not practical: when pyrrolidone lactam \textsuperscript{11} was treated with Rh\textsubscript{H}(CO)(PPh\textsubscript{3})\textsubscript{3} and Et\textsubscript{2}SiH\textsubscript{2}, the desired product \textsuperscript{12} was obtained in only 23\% yield after acid treatment (1 M hydrochloric acid) followed by regeneration of \textit{N}-Boc amine (Boc\textsubscript{2}O) (Scheme 5). The method by way of imidate shown in Scheme 4 is, therefore, more practical than borane reduction,\textsuperscript{11} or hydrosilylation, for deoxygenation of the pyrrolidone ring leading to advanced intermediates, \textit{N}-Boc-protected pyrrolidine \textbf{6a}–\textbf{6d}.\textsuperscript{19}

During the deoxygenation studies, the \textit{N}-Ac group was found to be unstable under reaction conditions amenable to imidate formation. For example, when \textbf{13} was subjected to Meerwein reagent and K\textsubscript{2}CO\textsubscript{3}, the desired imidate formation cleanly proceeded while deacetylation was partially observed (Scheme 6).\textsuperscript{20} Thus, pyrrolidines \textbf{14} and \textbf{15} were obtained as a mixture in 88\% yield. The undesirable side reaction was not observed with \textit{N}-TFA compound \textbf{10d} (Scheme 4), however, and we therefore expect that TFA amide could be generally stable under Meerwein conditions.

With the advanced intermediates \textbf{6a}–\textbf{6d}, four glutamate analogs bearing an olefin functionality at the C ring were directly synthesized by global deprotection of all protecting groups under acidic hydrolysis conditions (6 M hydrochloric acid, 65 °C) as shown in Scheme 7.\textsuperscript{8} After reversed-phase column chromatography using water as an eluant, the artificial glutamate analogs \textbf{7a}–\textbf{7d} were obtained cleanly in 79–100\% yield without any detectable by-products.

Next, glutamate analogs \textbf{5a}–\textbf{5d} saturated at the C ring were synthesized as shown in Scheme 8. Four advanced intermediates \textbf{6a}–\textbf{6d} were hydrogenated (H\textsubscript{2}, 10\% Pd/C, ca 3 h) to give \textbf{16a}–\textbf{16d} in excellent yields (96–100\%). Chromatographic and spectroscopic data of \textbf{16a} and \textbf{16b} were completely identical with those of compounds obtained in the first-generation synthesis.\textsuperscript{11} Finally, all protecting groups were simultaneously removed (6 M hydrochloric acid, 65 °C) to furnish \textbf{5a}–\textbf{5d} in 63–100\% yield. Again, \textbf{5a} and \textbf{5b} were identical in all respects with specimens prepared by our first-generation synthesis.\textsuperscript{11}

The dihydroxylated glutamate analogs \textbf{1a}–\textbf{1d} were synthesized as shown in Scheme 9. Os\textsubscript{O\textsubscript{4}}-induced dihydroxylation of advanced intermediates \textbf{6a}–\textbf{6d} was performed using \textit{N}-methylmorpholine \textit{N}-oxide (NMO) as a co-oxidant to provide \textbf{17a}–\textbf{17d} quantitatively in all cases. Although the reason is not clear at present, yields for the dihydroxylation
were substantively improved from the first-generation synthesis,\textsuperscript{11} in which the transformation of pyrrolidones 4\textit{a} and 4\textit{b} to corresponding diols proceeded with 88% and 83% yields, respectively (see Scheme 1). Stereochemistry of 17\textit{a} and 17\textit{b} was determined after converting into known glutamate analogs 1\textit{a} and 1\textit{b},\textsuperscript{11} which showed dihydroxylation had took place from a convex β-side of the molecular skeleton. By analogy with 17\textit{a} and 17\textit{b}, diol group of 17\textit{c'} and 17\textit{d} was determined also as β-oriented.\textsuperscript{21} Global deprotection of all protecting groups was performed by acidic hydrolysis (6 M hydrochloric acid, 65 °C), giving rise to dihydroxylated artificial glutamate analogs 1\textit{a}–1\textit{d} in 74–94% yield after reversed-phase column chromatography. Analogs 1\textit{a} and 1\textit{b} were identical in all respects with those synthesized using the first-generation synthesis.\textsuperscript{11}

2.2. Biological evaluation of the artificial glutamate analogs

On some structurally diverse glutamate analogs thus synthesized in racemic form, biological evaluation was performed in vitro (radioligand binding assays and electrophysiological analyses with GluRs) and in vivo (mice behavioral assays) as follows. In our radioligand binding assays using rat brain synaptic membranes, we did not find evidence for binding of selected analogs (1\textit{a}, 1\textit{b}, 5\textit{a}, 5\textit{b}) to any subtype of ionotropic glutamate receptors (iGluR) – AMPA, KA, or NMDA – at a concentration of 1 × 10\textsuperscript{-5} M. On the other hand, current- and voltage-clump electrophysiological analysis from cultured hippocampal neurons revealed that one of the new compounds (7\textit{c}) markedly reduced both action potential firing frequency, by 52 ± 9% (\textit{n}=3, \textit{p}<0.05) and charge transfer during spontaneous excitatory synaptic currents, by 31 ± 9% (\textit{n}=3), which was somewhat less than was observed with glutamate analog 1\textit{a}.\textsuperscript{11,12} Pharmacological characterization of related compound, being named IKM-159, suggests that these activities arise through specific inhibition of AMPA receptors, although the precise mechanism of action remains to be delineated.\textsuperscript{22}

The effect of the several compounds were examined in mouse behavioral assays following intracranial injection of each compound (20 µg/mouse).\textsuperscript{23} Compounds 1\textit{b}, 5\textit{a}, 5\textit{b}, and 7\textit{b} were hyperactive while other analogs were hypoactive.\textsuperscript{24} It was somewhat surprising to discover glutamate analogs with hypoactivity in this artificial compound library, since naturally derived glutamates such as dysiherbaine,\textsuperscript{5} neodysiherbaine,\textsuperscript{6} and kainic acid,\textsuperscript{25} are potent convulsants. Among those exhibiting hypoactivity, the piperidine-containing analogs were potent in the assay (1\textit{c} ≈ 5\textit{c} > 7\textit{c} order of potency);
upon intracranial injection (20 \(\mu g/mouse\)), mice developed head tremors accompanied by scratching behavior, and then went into state of immobility lasting for about 50 min. It should be noted that even the new unsaturated analog 7c, while producing a weaker form of hypoactivity than the other two piperidine-containing analogs, was clearly more potent than 1a, for which we recently reported similar biological activity.\textsuperscript{11,12} The behavioral hypoactivity of 7c also correlates with the reduced neuronal excitability observed in the in vitro assays, demonstrating that the pharmacological activity of these molecules differs substantively from their progenitor convulsant molecules, kainate and neodysiberaine A.

3. Conclusions

In conclusion, we have developed an improved, second-generation route amenable to syntheses of twelve artificial glutamate analogs (1a–1d, 5a–5d, 7a–7d) starting from 3a–3d, readily prepared in three steps. The synthesis features four advanced intermediates 6a–6d at 1–2 steps before the final products in the synthetic scheme, so that the glutamate analogs can be prepared diversely and efficiently. Twelve analogs were thus furnished in 7.2–25.8\% overall yields, which were clearly improved from the first-generation route for the synthesis of 1a, 1b, 5a, and 5b (4.3–16.3\% yields, see Scheme 1). Although the total steps are longer in the present study (13–15 steps) than those in the first-generation route (11–12 steps), the new route is capable of synthesizing new analogs bearing amino group and/or olefin functionality at the C ring.

Biological evaluation of a subset of the glutamate analogs showed diverse activities in vitro and in vivo. In particular, the three new piperidine-containing analogs (1c, 5c, 7c) were discovered to be more hypoactive than the previously reported 1a. In the case of 7c, this in vivo hypoactivity was matched by inhibitory actions on neuronal excitability and synaptic transmission in vitro. Further biological studies are under progress to establish the structure-activity relationships and to improve the biological potency, and the results will be reported in due course.

4. Experimental

4.1. General

The experimental techniques and the characterizing apparatuses used are summarized
in our previous paper.8 Electrophysiological experiments were performed according to our published procedure.11 For procedures and data for intermediates 16a and 16b, and glutamate analogs 1a, 1b, 5a, and 5b, see our first-generation synthesis paper.11

4.1.1.
\(1\E-2-(3S*,3aS*,3bS*,3cS*,4aR*,8aR*,8bR*)-8-Aza-3-((N\text{-}benzyl-N\text{-}tert-butoxycarbonyl)carbamoyl)-2-(4-methoxybenzyl)-8-(2-nitrobenzenesulfonyl)-2,3,3a,4a,7,8,8a,8b-octahydro-1-oxo-1H-benzofuro[2,3-c]pyrrol-3a-yl)vinyl acetate (8c).

To a stirred solution of the N-Bn amide 3c (1.00 g, 1.42 mmol) in DCM (15 mL) at 0 °C were added Boc\(_2\)O (1.66 mL, 7.10 mmol), TEA (984 µL, 7.10 mmol) and DMAP (86.7 mg, 0.71 mmol). After 2.5 h, the mixture was diluted with EtOAc (50 mL), washed with saturated aqueous NH\(_4\)Cl (50 mL) and brine (50 mL), dried over Na\(_2\)SO\(_4\), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 7:3) to give the N-Boc imide 8c (1.00 g, 90%) as a pale yellow solid: IR (film) 2930, 1696, 1544, 1370, 1147 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 8.52 (d, \(J = 8.0\) Hz, 1 H), 7.78 (t, \(J = 8.0\) Hz, 1 H), 7.71 (t, \(J = 8.0\) Hz, 1 H), 7.65 (d, \(J = 8.0\) Hz, 1 H), 7.25–7.13 (m, 6 H), 6.94 (d, \(J = 9.0\) Hz, 2 H), 6.77 (d, \(J = 9.0\) Hz, 2 H), 5.86 (dd, \(J = 10.5\) Hz, 1 H), 5.78 (d, \(J = 10.5\) Hz, 1 H), 5.30 (d, \(J = 12.5\) Hz, 1 H), 5.25 (s, 1 H), 5.02 (dd, \(J = 7.5\), 2.5 Hz, 1 H), 4.82 (d, \(J = 14.5\) Hz, 1 H), 4.76 (d, \(J = 7.5\) Hz, 1 H), 4.70 (d, \(J = 14.0\) Hz, 1 H), 4.48 (d, \(J = 14.5\) Hz, 1 H), 4.26 (dd, \(J = 18.0\), 5.5 Hz, 1 H), 3.75 (s, 3 H), 3.75 (d, \(J = 14.0\) Hz, 1 H), 3.46 (d, \(J = 18.0\) Hz, 1 H), 3.12 (s, 1 H), 2.02 (s, 3 H), 1.28 (s, 9 H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 172.9, 171.8, 167.2, 159.2, 151.8, 148.0, 138.2, 138.1, 133.8, 132.7, 132.5, 132.1, 130.1 \((\times 2)\), 128.2 \((\times 2)\), 128.1 \((\times 2)\), 127.9, 127.4, 126.4, 126.1, 123.9, 114.1 \((\times 2)\), 113.0, 84.7, 84.4, 72.3, 68.9, 58.4, 55.8, 55.2, 47.6, 45.8, 40.2, 27.6 \((\times 3)\), 20.5; HRMS (ESI, positive) calcd for C\(_{40}\)H\(_{43}\)N\(_4\)O\(_{12}\)S \([M+H]^+\) 803.2593, found 803.2591.

4.1.2.
\(1\E-2-((Z,1S*,3aR*,3bS*,8aS*,9aS*)-4-Aza-1-((N\text{-}benzyl-N\text{-}tert-butoxycarbonyl)carbamoyl)-2-(4-methoxybenzyl)-9-oxa-4-trifluoroacetyl-2,3,3a,4b,4b,5,6,8a,9a-decahydro-3\text{-}oxo-1H-azuleno[2,1-c]pyrrol-9a-yl)vinyl acetate (8d).

With the same procedure for the synthesis of 8c, 8d (351.3 mg, 83%) was obtained as a pale yellow solid starting from 3d (363.6 mg, 0.58 mmol), Boc\(_2\)O (407.9 mg, 1.74 mmol), DMAP (35.4 mg, 0.29 mmol), and TEA (241.1 µL, 1.74 mmol).
Data for 8d. IR (film) 2894, 1702, 1513, 1210, 1146, 848 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, ca 1:1 mixture of rotamers) δ 7.32 (d, J = 13.0 Hz, 0.5 H), 7.29–7.19 (m, 5.5 H), 6.97–6.94 (m, 2 H), 6.79–6.78 (d, J = 8.0 Hz, 2 H), 5.94–5.86 (m, 0.5 H), 5.70–5.62 (m, 0.5 H), 5.47 (d, J = 13.0 Hz, 0.5 H), 5.45 (d, J = 13.0 Hz, 0.5 H), 5.36 (s, 0.5 H), 5.35 (s, 0.5 H), 5.18 (m, 0.5 H), 4.90–4.83 (m, 3.5 H), 4.58 (d, J = 14.5 Hz, 0.5 H), 4.54 (d, J = 14.5 Hz, 0.5 H), 3.95–3.87 (m, 1 H), 3.76 (s, 3 H), 3.73–3.62 (m, 2 H), 3.22 (d, J = 13.0 Hz, 0.5 H), 2.67 (m, 0.5 H), 2.47–2.22 (m, 1 H), 2.05 (s, 3 H), 1.34–1.28 (m, 9 H); ¹³C NMR (125 MHz, CDCl₃, selected) δ 171.6, 171.1, 167.2, 159.3, 159.3, 156.8, 151.7, 139.1, 137.3, 137.0, 130.3 (× 2), 128.0 (× 2), 127.4 (× 2), 121.7, 120.9, 117.4, 114.1 (× 2), 84.7, 83.4, 76.1, 70.1, 64.2, 60.9, 60.5, 47.7, 45.5, 41.6, 41.5, 32.6, 27.5 (× 3), 20.5; HRMS (ESI, positive) calcd for C₃₇H₄₁N₃O₉F₃ [(M+H)⁺] 728.2789, found 728.2787.

4.1.3. Methyl (3⁵S,3a⁵S,4aR⁵*,8aR⁵*,8bR⁵*) 8-aza-2-(4-methoxybenzyl)-3a-formylmethyl-8-(2-nitrobenzensulfonyl)-2,3,3a,4a,7,8,8a,8b-octahydro-1-oxo-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (9c).

To a stirred solution of the imide 8c (1.03 g, 1.28 mmol) in methanol (45 mL) at –20 °C was added K₂CO₃ (88.3 mg, 0.64 mmol). After 5 h, the mixture was poured into saturated aqueous NH₄Cl (60 mL), and the mixture was extracted with EtOAc (100 mL). The extract was washed with brine (60 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (6 g, hexane/EtOAc = 7:3) to give the ester aldehyde 9c (604.4 mg, 81%) as a pale yellow solid: IR (film) 3002, 1748, 1698, 1541, 1508, 1362, 1248, 1172, 1030, 582 cm⁻¹; ¹H NMR (500 MHz, C₆D₆) δ 9.44 (s, 1 H), 8.44 (d, J = 8.0 Hz, 1 H), 7.02–6.97 (m, 3 H), 6.56 (t, J = 7.5 Hz, 1 H), 5.18 (ddd, J = 10.8, 2.0, 2.0 Hz, 1 H), 5.10 (ddd, J = 10.8, 3.5, 3.5 Hz, 1 H), 4.93 (d, J = 14.5 Hz, 1 H), 4.76 (dd, J = 6.8, 3.5 Hz, 1 H), 4.28 (s, 1 H), 4.21 (br s, 1 H), 3.92 (d, J = 14.5 Hz, 1 H), 3.92 (d, J = 18.0 Hz, 1 H), 3.51 (d, J = 4.0 Hz, 1 H), 3.48 (d, J = 14.5 Hz, 1 H), 3.24 (m, 1 H), 3.23 (s, 3 H), 3.11 (s, 3 H), 2.71 (dd, J = 17.5, 1.0 Hz, 1 H), 2.31 (dd, J = 17.5, 1.0 Hz, 1 H); ¹³C NMR (125 MHz, C₆D₆) δ 197.7, 171.8, 169.5, 159.9, 148.8, 133.8, 132.5, 131.6, 131.2, 130.1 (× 2), 127.4, 127.3, 125.5, 123.8, 114.6 (× 2), 84.6, 73.7, 68.9, 59.5, 54.7, 54.4, 51.8, 48.7, 45.5, 41.5; HRMS (ESI, positive) calcd for C₂₇H₄₁N₃O₁₀S [(M+H)⁺] 586.1489, found 586.1490.

4.1.4. Methyl (Z,15⁵S,3aR⁵*,3bS⁵*,8aS⁵*,9aS⁵*) 4-aza-2-(4-methoxybenzyl)-9a-formylethyl-9-oxa-4-trifluoroacetyl-2,3,3a,3b,4,5,6,8a,9,9a-decahydro-3-oxo-1H-azuleno[2,1-c]pyrrole-1-carboxylate (9d).
With the same procedure for the synthesis of 9c, 9d (219.4 mg, 91%) was obtained as a pale yellow solid starting from 8d (345.0 mg, 0.48 mmol) and K₂CO₃ (32.8 mg, 0.24 mmol).

**Data for 9d.** IR (film) 2933, 1748, 1698, 1541, 1508, 1145, 669 cm⁻¹; ¹H NMR (500 MHz, CD6, ca 1:1 mixture of rotamers) δ 9.58 (br s, 0.5 H), 9.55 (br s, 0.5 H), 6.99 (d, J = 8.5 Hz, 1 H), 6.94 (d, J = 8.5 Hz, 1 H), 6.66 (d, J = 8.5 Hz, 1 H), 6.62 (d, J = 8.5 Hz, 1 H), 5.31–5.25 (m, 1 H), 5.16–5.02 (m, 1 H), 4.89 (d, J = 5.5 Hz, 0.5 H), 4.21 (s, 0.5 H), 4.08 (s, 0.5 H), 4.06 (dd, J = 7.3, 5.0 Hz, 0.5 H), 3.93 (dd, J = 7.3, 5.0 Hz, 0.5 H), 3.82–3.74 (m, 1.5 H), 3.41 (t, J = 13.5 Hz, 0.5 H), 3.31–3.22 (m, 6 H), 3.15 (s, 1.5 H), 3.10 (t, J = 12.0 Hz, 0.5 H), 2.90 (s, 0.5 H), 2.64 (br s, 0.5 H), 2.45–2.38 (m, 1.5 H), 2.35–2.32 (m, 1 H), 1.78 (br t, J = 18.0 Hz, 0.5 H), 1.66 (br d, J = 20.0 Hz, 0.5 H), 1.50 (br d, J = 20.0 Hz, 0.5 H), 0.91 (m, 0.5 H); ¹³C NMR (125 MHz, CD6, selected) δ 197.6, 170.4, 169.8, 161.1, 137.1, 130.6, 128.8 (× 2), 127.5, 122.0, 118.3, 114.8 (× 2), 82.9, 76.8, 69.5, 65.9, 65.0, 54.9, 52.1, 45.7, 42.1, 32.8, 29.9; HRMS (ESI, positive) calcd for C₂₄H₂₅F₃N₂O₇Na [(M+Na)⁺] 533.1506, found 533.1493.

4.1.5. Methyl (3S*,3aS*,4aR*,8aR*,8bR*)-8-aza-3a-((methoxycarbonyl)methyl)-2-(4-methoxybenzyl)-8-(2-nitrobenzenesulfonyl)-2,3,3a,4a,7,8,8a,8b-octahydro-1-oxo-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (4c).

To a stirred solution of the aldehyde 9c (604.4 g, 1.03 mmol) in tert-butanol (36 mL) and water (12 mL) at rt were added 2-methyl-2-butene (547.0 µL, 5.16 mmol), NaH₂PO₄·2H₂O (177.0 mg, 1.13 mmol), and NaClO₂ (278 mg, 3.09 mmol). After 5 h, the mixture was diluted with DCM (100 mL), and the mixture was washed with hydrochloric acid (1 M, 50 mL) and brine (30 mL). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The residue was dissolved in methanol (36 mL) and cooled to 0 °C. TMSCHN₂ (2 M in Et₂O, 1.03 mL, 2.06 mmol) was added, and the mixture was allowed to warm to rt. After stirring for 30 min, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (15 g, hexane/EtOAc = 4:6) to give the diester 4c (589.6 mg, 93%) as a white solid: IR (film) 2953, 1745, 1699, 1544, 1513, 1248, 1172, 1031, 682 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 8.36 (dd, J = 7.5, 1.0 Hz, 1 H), 7.82–7.68 (m, 3 H), 7.12 (d, J = 8.5 Hz, 2 H), 6.88 (d, J = 8.5 Hz, 2 H), 6.00 (dt, J = 11.0, 4.0 Hz, 1 H), 5.80 (dd, J = 11.0, 3.0 Hz, 1 H), 4.94 (d, J = 14.5 Hz, 1 H), 4.70 (t, J = 7.5 Hz, 1 H), 4.63 (br s, 1 H), 4.19 (s, 1 H), 4.10 (dt, J = 16.0, 2.0 Hz, 1 H), 3.94 (d, J = 14.5 Hz, 1 H), 3.91 (d, J = 16.0 Hz, 1 H), 3.82 (dd, J = 11.0, 4.0 Hz, 1 H), 3.64 (dd, J = 11.0, 3.0 Hz, 1 H), 3.56 (s, 1 H), 3.49 (dt, J = 16.0, 2.0 Hz, 1 H), 3.44 (d, J = 14.5 Hz, 1 H), 2.98 (br s, 1 H), 2.62 (dd, J = 11.0, 3.0 Hz, 1 H), 2.38 (s, 1 H), 2.30 (t, J = 12.0 Hz, 1 H), 2.18 (t, J = 12.0 Hz, 1 H), 1.83 (br s, 1 H), 1.68 (br s, 1 H), 1.60 (br s, 1 H), 1.50 (br s, 1 H), 1.35 (br s, 1 H), 1.28 (s, 3 H), 1.24 (s, 3 H), 1.17 (s, 3 H), 1.10 (s, 3 H), 1.07 (s, 3 H), 0.98 (s, 3 H), 0.93 (s, 3 H), 0.90 (s, 3 H), 0.88 (s, 3 H), 0.86 (s, 3 H), 0.84 (s, 3 H), 0.82 (s, 3 H), 0.80 (s, 3 H), 0.78 (s, 3 H), 0.75 (s, 3 H), 0.73 (s, 3 H), 0.71 (s, 3 H), 0.69 (s, 3 H), 0.67 (s, 3 H), 0.65 (s, 3 H), 0.63 (s, 3 H), 0.61 (s, 3 H), 0.59 (s, 3 H), 0.57 (s, 3 H), 0.55 (s, 3 H), 0.53 (s, 3 H), 0.51 (s, 3 H), 0.49 (s, 3 H), 0.47 (s, 3 H), 0.45 (s, 3 H), 0.43 (s, 3 H), 0.41 (s, 3 H), 0.39 (s, 3 H), 0.37 (s, 3 H), 0.35 (s, 3 H), 0.33 (s, 3 H), 0.31 (s, 3 H), 0.29 (s, 3 H), 0.27 (s, 3 H), 0.25 (s, 3 H), 0.23 (s, 3 H), 0.21 (s, 3 H), 0.19 (s, 3 H), 0.17 (s, 3 H), 0.15 (s, 3 H), 0.13 (s, 3 H), 0.11 (s, 3 H), 0.09 (s, 3 H), 0.07 (s, 3 H), 0.05 (s, 3 H), 0.03 (s, 3 H), 0.01 (s, 3 H), 0.00 (s, 3 H), 0.00 (s, 3 H).
Hz, 1 H), 3.82 (s, 3 H), 3.70 (d, J = 5.0 Hz, 1 H), 3.67 (s, 3 H), 3.64 (s, 3 H), 2.97 (d, J = 16.5 Hz, 1 H), 2.82 (d, J = 16.5 Hz, 1 H); 13C NMR (125 MHz, CDCl3) δ 171.5, 169.3 (× 2), 159.3, 148.2, 134.1, 132.2, 132.0, 131.4, 129.8 (× 2), 126.8, 126.7, 125.9, 124.2, 114.2 (× 2), 84.7, 73.1, 63.4, 58.7, 55.2, 52.8, 52.5, 51.9, 45.2, 40.9, 39.7: HRMS (ESI, positive) calcd for C28H29N3O11SNa [(M+Na)+] 638.1415, found 638.1392.

4.1.6. Methyl (Z,1S*,3aR*,3bS*,8aS*,9aS*) 4-aza-9a-(methoxycarbonyl)methyl-2-(4-methoxybenzyl)-9-oxa-4-trifluoroacetyl-2,3,3a,4,5,6,8a,9,9a-decahydro-3-oxo-1H-azuleno[2,1-c]pyrrole-1-carboxylate (4d).

With the same procedure for the synthesis of 4c, 4d (183.7 mg, 85%) was obtained as a pale yellow oil starting from 9d (210.4 mg, 0.41 mmol), 2-methyl-2-butene (218.0 µL, 2.06 mmol), NaH2PO4·2H2O (70.7 mg, 0.46 mmol), NaClO2 (111.3 mg, 1.24 mmol), and TMSCHN2 (2 M in Et2O, 0.41 mL, 0.81 mmol).

Data for 4d. IR (film) 2954, 1745, 1702, 1513, 1438, 1205, 1167, 1035 cm−1; 1H NMR (500 MHz, CDCl3, ca 6:4 mixture of rotamers) δ 7.12–7.01 (m, 2 H), 6.85–6.82 (m, 2 H), 5.98–5.90 (m, 1 H), 5.60–5.54 (m, 1 H), 5.07 (d, J = 14.5, 0.4 H), 5.00 (d, J = 14.5 Hz, 0.6 H), 4.99 (d, J = 5.5 Hz, 0.6 H), 4.72 (d, J = 5.5 Hz, 0.4 H), 4.15 (s, 0.6 H), 4.11–4.03 (m, 1.4 H), 3.90–3.85 (m, 2 H), 3.79–3.78 (m, 3 H), 3.70 (s, 1.2 H), 3.66–3.64 (m, 4.8 H), 3.56–3.51 (m, 0.6 H), 3.43 (s, 0.4 H), 3.37 (s, 0.6 H), 2.98–2.94 (m, 1.4 H), 2.82–2.69 (m, 1 H), 2.50–2.31 (m, 2 H); 13C NMR (125 MHz, CDCl3, major rotamer) δ 170.3, 169.4, 168.9, 159.3, 137.0, 130.0 (× 2), 126.9, 121.6, 120.5, 114.1 (× 2), 82.8, 75.7, 68.4, 64.6, 58.6, 55.2, 52.4, 52.0, 45.1, 41.6, 39.9, 32.7, 29.6: HRMS (ESI, positive) calcd for C25H27N2O8F3Na [(M+Na)+] 563.1612, found 563.1609.

4.1.7. Methyl (3aS*,3bR*,4aR*,8aR*,8bR*) 3a-(methoxycarbonylmethyl)-8-oxa-2,3,3a,4a,7,8a,8b-octahydro-1-oxo-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (10a).

To a stirred solution of the N-PMB amide 4a (187.3 mg, 0.43 mmol) in CH3CN (10.0 mL) and water (2.4 mL) at −10 °C was added a solution of CAN (1.19 mg, 2.17 mmol) in water (6.6 mL) portionwise. After 5 h, the mixture was poured into saturated aqueous Na2S2O3 (20 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with saturated aqueous NaHCO3 (20 mL) and brine (20 mL), dried over Na2SO4, and concentrated under reduced pressure. The residue was purified by column
chromatography on silica gel (5 g, hexane/EtOAc = 4:6) to give the lactam 10a (95.2 mg, 71%) as a white solid: IR (film) 2953, 1747, 1698, 1508, 1436, 1250, 1211, 1087, 848, 688 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.16 (s, 1 H), 6.05 (dd, J = 10.0, 4.0 Hz, 1 H), 5.98 (ddd, J = 16.5, 4.0 Hz, 1 H), 4.44 (s, 1 H), 4.29 (d, J = 2.0 Hz, 1 H), 4.13 (dd, J = 16.5 Hz, 1 H), 3.67 (s, 3 H), 3.60 (s, 3 H), 3.28 (s, 1 H), 3.14 (d, J = 17.5 Hz, 1 H), 3.03 (s, J = 17.5 Hz, 1 H); ¹³C NMR (125 MHz, CDCl₃) δ 174.1, 170.4, 170.3, 130.9, 122.0, 87.3, 78.1, 73.2, 64.7, 64.1, 56.7, 52.5, 51.6, 40.3; HRMS (ESI, positive) calcd for C₁₄H₁₇NO₇Na [(M+Na)+] 334.0897, found 334.0899.

4.1.8. Methyl (Z,1S*,3aR*,3bS*,8aS*,9aS*) 9a-((methoxycarbonyl)methyl)-2,3,3a,3b,4,5,6,8a,9,9a-decahydro-4,9-dioxa-3-oxo-1H-azulenol[2,1-c]pyrrole-1-carboxylate (10b).

With the same procedure for the synthesis of 10a, 10b (67.4 mg, 71%) was obtained as a white solid starting from 4b (130 mg, 0.29 mmol) and CAN (801 mg, 1.46 mmol).

**Data for 10b.** IR (film) 2953, 1743, 1715, 1436, 1362, 1211, 1046, 669 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.92 (br s, 1 H), 5.81 (dd, J = 11.5, 5.0, 5.0 Hz, 1 H), 5.64 (dd, J = 11.5, 2.5 Hz, 1 H), 4.54 (br s, 1 H), 4.40 (s, 1 H), 4.37 (br s, 1 H), 3.96 (dd, J = 11.5, 5.5, 4.5 Hz, 1 H), 3.69 (s, 3 H), 3.64 (s, 3 H), 3.62 (dd, J = 11.5, 5.5, 4.5 Hz, 1 H), 3.29 (s, 1 H), 3.22 (d, J = 17.5 Hz, 1 H), 2.92 (d, J = 17.5 Hz, 1 H), 2.35 (dd, J = 5.5, 5.0 Hz, 2 H); ¹³C NMR (125 MHz, CDCl₃) δ 174.6, 170.4, 170.3, 129.7, 126.1, 85.8, 82.5, 82.0, 68.9, 64.6, 58.0, 52.5, 51.6, 39.6, 30.3; HRMS (ESI, positive) calcd for C₁₅H₁₉NO₇Na [(M+Na)+] 348.1054, found 348.1060.

4.1.9. Methyl (3S*,3aS*,4aR*,8aS*,9aS*) 8-aza-3a-((methoxycarbonyl)methyl)-8-(2-nitrobenzenesulfonyl)-2,3,3a,4a,7,8,8a,8b-octa hydro-1-oxo-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (10c).

With the same procedure for the synthesis of 10a, 10c (178.4 mg, 78%) was obtained as a white solid starting from 4c (278.4 mg, 0.45 mmol) and CAN (1.24 g, 2.26 mmol).

**Data for 10c.** IR (film) 2922, 1715, 1541, 1362, 1253, 1166, 683, 584 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 8.19 (dd, J = 5.5, 4.5 Hz, 1 H), 7.72–7.64 (m, 3 H), 6.02 (br s, 1 H), 5.91 (dd, J = 10.0, 1.0 Hz, 1 H), 5.74 (d, J = 10.0 Hz, 1 H), 4.91 (t, J = 7.5 Hz, 1 H), 4.80 (br s, 1 H), 4.41 (s, 1 H), 4.13 (d, J = 19.0 Hz, 1 H), 3.87 (d, J = 19.0 Hz, 1 H), 3.75 (s, 3 H), 3.59 (s,
3 H), 3.37 (d, J = 7.5 Hz, 1 H), 3.05 (d, J = 16.5 Hz, 1 H), 2.81 (d, J = 16.5 Hz, 1 H); 13C NMR (125 MHz, CDCl$_3$) $\delta$ 173.2, 169.3, 169.2, 148.0, 134.0, 132.4, 132.1, 131.9, 126.4, 126.3, 124.5, 87.5, 73.7, 65.6, 58.0, 52.8, 52.0, 50.8, 40.8, 40.1: HRMS (ESI, positive) calcd for C$_{20}$H$_{22}$N$_{3}$O$_{10}$S [(M+H)$^+$] 496.1020, found 496.1020.

4.1.10. Methyl (Z,1$^*$,3a$^*$,3b$^*$,8a$^*$,9a$^*$) 4-aza-9a-(((methoxycarbonyl)methyl)-9-oxa-4-trifluoroacetyl-2,3,3a,4,5,6,8a,9,9a-decahydro-3-oxo-1H-azuleno[2,1-c]pyrrole-1-carboxylate (10d).

With the same procedure for the synthesis of 10a, 10d (109.4 mg, 80%) was obtained as a pale yellow solid starting from 4d (175.5 mg, 0.33 mmol) and CAN (891 mg, 1.63 mmol).

Data for 10d. IR (film) 2930, 1716, 1436, 1209, 1146, 1046, 730 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$, ca 1:1 mixture of rotamers) $\delta$ 6.15 (br s, 0.5 H), 6.09 (br s, 0.5 H), 6.03–5.97 (m, 1 H), 5.78–5.72 (m, 1 H), 5.04 (d, J = 6.0 Hz, 0.5 H), 4.75 (d, J = 6.0 Hz, 0.5 H), 4.49–4.45 (m, 1 H), 4.29 (s, 0.5 H), 4.24 (s, 0.5 H), 4.06 (dd, J = 13.5, 4.5 Hz, 0.5 H), 3.85 (d, J = 8.0 Hz, 1 H), 3.77 (s, 1.5 H), 3.76 (s, 1.5 H), 3.64 (s, 1.5 H), 3.63 (s, 1.5 H), 3.45 (ddd, J = 12.5, 10.5, 3.5 Hz, 0.5 H), 3.40 (d, J = 17.0 Hz, 0.5 H), 3.25–3.24 (m, 1 H), 3.24 (d, J = 17.0 Hz, 0.5 H), 2.96 (d, J = 17.0 Hz, 0.5 H), 2.94 (d, J = 17.0 Hz, 0.5 H), 2.82 (m, 0.5 H), 2.57–2.36 (m, 1.5 H); $^{13}$C NMR (125 MHz, CDCl$_3$, selected) $\delta$ 173.4, 172.9, 169.6, 169.3, 137.7, 120.3, 85.3, 75.3, 64.8, 63.6, 59.2, 52.7, 52.0, 41.2, 39.8, 32.3, 29.1: HRMS (ESI, positive) calcd for C$_{17}$H$_{19}$N$_{2}$O$_{7}$Na [(M+Na)$^+$] 443.1037, found 443.1038.

4.1.11. Methyl (3$^*$,3a$^*$,4a$^*$,8a$^*$,9b$^*$) 2-(tert-butoxycarbonyl)-3a-((methoxycarbonyl)methyl)-8-oxa-2,3,3a,4a,7,8,8a,8b-octahydro-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (6a).

To a stirred solution of the pyrrolidinone 10a (65.8 mg, 0.212 mmol) in DCM (2.0 mL) at 0 °C were added Me$_3$O·BF$_4$ (94.1 mg, 0.636 mmol) and K$_2$CO$_3$ (117.2 mg, 0.848 mmol). After stirring at rt for 4 h, the mixture was diluted with DCM (20 mL), washed with water (10 mL) and brine (10 mL), dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The crude imidate thus obtained was used in the next reaction without purification.

To a stirred solution of the above imidate in methanol (2.0 mL) at 0 °C were added
NaCNBH₃ (40 mg, 0.636 mmol) and TFA (31.5 µL, 0.424 mmol). After stirring at rt for 4 h, the mixture was diluted with DCM (20 mL), washed with saturated aqueous NaHCO₃ (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure to a final volume of ca 2 mL. Boc₂O (149 µL, 0.636 mmol) and TEA (88 µL, 0.636 mmol) were added, and the mixture was stirred at rt for 2 h. The mixture was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (3 g, hexane/EtOAc = 8:2) to give the pyrrolidine 6a (49.4 mg, 59 %, 3 steps) as a white solid: IR (film) 1742, 1701, 1395, 1366, 1174, 1013, 689 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, ca 7:3 mixture of rotamers) δ 6.01 (m, 2 H), 4.74 (s, 1 H), 4.29 (br s, 0.7 H), 4.26 (br s, 0.3 H), 4.15 (d, J = 16.5 Hz, 1 H), 3.98 (d, J = 16.5 Hz, 1 H), 3.94–3.90 (m, 2 H), 3.66 (s, 4.2 H), 3.65 (s, 1.8 H), 3.37 (dd, J = 10.0, 4.5 Hz, 0.7 H), 3.32 (m, 0.3 H), 3.17 (d, J = 17.0 Hz, 0.7 H), 3.14 (d, J = 17.0 Hz, 0.3 H), 3.07 (br d, J = 6.0 Hz, 0.3 H), 3.00 (dd, J = 10.0, 4.5 Hz, 0.7 H), 2.68 (d, J = 17.0 Hz, 0.3 H), 2.62 (d, J = 17.0 Hz, 0.7 H), 1.38 (s, 9 H); ¹³C NMR (125 MHz, CDCl₃, selected) δ 171.0, 170.3, 154.0, 130.5, 122.7, 92.0, 81.1, 80.4, 73.0, 69.0, 64.0, 52.0, 51.6, 51.5, 48.7, 40.4, 28.2 (× 3): HRMS (ESI, positive) calcd for C₁₉H₂₇N₁O₈Na [(M+Na)+] 420.1629, found 420.1622.

4.1.12. Methyl \((Z,1S^*,3aR^*,3bS^*,8aS^*,9aS^*)\) 2-(tert-butoxycarbonyl)-9a-((methoxycarbonyl)methyl)-4,9-dioxa-2,3,3a,3b,4,5,6,8a,9,9a-decahydro-1H-azuleno[2,1-c]pyrrole-1-carboxylate (6b).

With the same procedure for the synthesis of 6a, 6b (10.4 mg, 85%) was obtained as a white solid starting from 10b (9.7 mg, 0.030 mmol), Me₃O BF₄ (13.2 mg, 0.090 mmol), and NaCNBH₃ (5.62 mg, 0.089 mmol).

Data for 6b. IR (film) 1745, 1701, 1396, 1171, 669 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, ca 7:3 mixture of rotamers) δ 5.76 (dt, J = 12.0, 4.5 Hz, 1 H), 5.67 (dd, J = 12.0, 4.5 Hz, 1 H), 4.68–4.64 (br s, 2 H), 3.95–3.88 (m, 3 H), 3.71–3.61 (m, 6 H), 3.52–3.48 (m, 1 H), 3.32 (m, 1 H), 3.16 (d, J = 17.0 Hz, 0.7 H), 3.12 (d, J = 17.0 Hz, 0.3 H), 3.05 (dd, J = 10.0, 5.0 Hz, 0.3 H), 2.97 (dd, J = 10.0, 5.0 Hz, 0.7 H), 2.67 (d, J = 17.0 Hz, 0.3 H), 2.60 (d, J = 17.0 Hz, 0.7 H), 2.36–2.26 (m, 2 H), 1.37 (s, 9 H); ¹³C NMR (125 MHz, CDCl₃, selected) δ 171.0, 170.3, 154.0, 129.5, 126.8, 90.5, 85.5, 82.1, 80.4, 68.9, 68.8, 52.9, 52.0, 51.5, 49.5, 39.7, 30.6, 28.2 (× 3): HRMS (ESI, positive) calcd for C₂₀H₂₉NO₅Na [(M+Na)+] 434.1785, found 434.1788.

4.1.13. Methyl \((3S^*,3aS^*,4aR^*,8aR^*,8bR^*)\)
8-aza-2-(tert-butoxycarbonyl)-3a-((methoxycarbonyl)methyl)-8-(2-nitrobenzenesulfonyl)-2,3,3a,4a,7,8a,8b-octahydro-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (6c).

With the same procedure for the synthesis of 6a, 6c (162.1 mg, 86%) was obtained as a pale yellow solid starting from 10c (160.1 mg, 0.323 mmol), MesO·BF₄ (143.3 mg, 0.969 mmol), and NaCNBH₃ (101.5 mg, 1.615 mmol).

Data for 6c. IR (film) 2977, 1747, 1698, 1542, 1364, 1168, 682 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, ca 1:1 mixture of rotamers) δ 8.08 (d, J = 8.0 Hz, 0.5 H), 8.02 (d, J = 8.0 Hz, 0.5 H), 7.74–7.67 (m, 3 H), 5.81 (dd, J = 10.8, 3.5 Hz, 0.5 H), 5.75 (dd, J = 10.8, 3.5 Hz, 0.5 H), 5.65–5.62 (m, 1 H), 4.63 (d, J = 7.0 Hz, 0.5 H), 4.56 (d, J = 7.0 Hz, 0.5 H), 4.52 (s, 0.5 H), 4.51–4.45 (m, 1 H), 4.37 (s, 0.5 H), 4.15 (br d, J = 18.0 Hz, 0.5 H), 4.01 (d, J = 11.5 Hz, 0.5 H), 3.96 (br d, J = 18.0 Hz, 0.5 H), 3.81–3.77 (m, 1 H), 3.67 (s, 1.5 H), 3.62 (dd, J = 11.8, 5.5 Hz, 0.5 H), 3.59 (s, 3 H), 3.58 (d, J = 11.5 Hz, 0.5 H), 3.51 (dd, J = 11.8, 5.5 Hz, 0.5 H), 3.06 (m, 1 H), 2.83 (d, J = 15.5 Hz, 0.5 H), 2.77 (d, J = 15.5 Hz, 0.5 H), 2.72 (d, J = 15.5 Hz, 0.5 H), 2.67 (d, J = 15.5 Hz, 0.5 H), 1.46 (s, 4.5 H), 1.39 (s, 4.5 H); ¹³C NMR (125 MHz, CDCl₃, selected) δ 170.3, 169.8, 154.6, 147.8, 134.0, 132.4, 132.0, 131.0, 126.9, 124.7, 124.2, 90.7, 80.8, 71.6, 69.8, 58.8, 52.2, 51.8, 48.5, 46.7, 40.5, 39.7, 28.2 (× 3); HRMS (ESI, positive) calcd for C₂₅H₃₁N₃O₁₁Na [(M+Na)+] 604.1571, found 604.1566.

4.1.14. Methyl (Z,1S*,3aR*,3bS*,8aS*,9aS*)-4-aza-2-(tert-butoxycarbonyl)-9a-((methoxycarbonyl)methyl)-2-(4-methoxybenzyl)-9-oxa-4-trifluoroacetyl-2,3a,3b,4,5,6,8a,9,9a-decahydro-1H-azuleno[2,1-c]pyrrole-1-carboxylate (6d).

With the same procedure for the synthesis of 6a, 6d (110.1 mg, 84%) was obtained as a pale yellow solid starting from 10d (109.0 mg, 0.259 mmol), MesO·BF₄ (114.9 mg, 0.777 mmol), and NaCNBH₃ (48.8 mg, 0.777 mmol).

Data for 6d. IR (film) 2977, 1746, 1688, 1394, 1211, 1143, 731 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, ca 6:4 mixture of rotamers) δ 5.98–5.89 (m, 1 H), 5.75–5.68 (m, 1 H), 4.70 (s, 0.4 H), 4.54–4.50 (m, 1 H), 4.44–4.37 (m, 0.6 H), 4.01–3.98 (m, 1 H), 3.92–3.78 (m, 3 H), 3.71 (s, 3 H), 3.65 (s, 3 H), 3.45 (m, 0.6 H), 3.14 (m, 0.4 H), 3.00–2.97 (m, 1 H), 2.90–2.81 (m, 1.6 H), 2.73 (t, J = 16.0 Hz, 0.4 H), 2.49 (br s, 0.6 H), 2.42–2.30 (m, 1.4 H), 1.45 (br s, 3.6 H), 1.39 (s, 5.4 H); ¹³C NMR (125 MHz, CDCl₃, selected) δ 170.5, 169.6, 157.3, 153.3, 136.6,
HRMS (ESI, positive) calcd for C_{22}H_{29}N_{2}O_{8}Na [(M+Na)^+] 529.1768, found 529.1753.

4.1.15. Methyl \((3S,3aS,4aR,8aR,8bR)^*\)
\(8\text{-aza-2,8-bis(\text{tert}-\text{butoxycarbonyl})-3a\text{-}((\text{methoxycarbonyl})\text{methyl})-2,3,3a,4a,7,8,8a,8b\text{-octahydro-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (6c').}\)

To a stirred solution of \(6c\) (141.2 mg, 0.243 mmol) in CH\(_3\)CN (3.0 mL) at 0 °C were added thiophenol (49.8 \(\mu\)L, 0.485 mmol) and Cs\(_2\)CO\(_3\) (119.0 mg, 0.365 mmol). After stirring at rt for 1.5 h, the mixture was diluted with chloroform (50 mL), washed with saturated aqueous NaHCO\(_3\) (25 mL), dried over Na\(_2\)SO\(_4\), and concentrated under reduced pressure to a final volume of ca 3 mL. Boc\(_2\)O (170.9 \(\mu\)L, 0.729 mmol) and pyridine (59 \(\mu\)L, 79.1 mmol) were added, and the mixture was stirred at rt for 2 h. The mixture was then diluted with DCM (50 mL), washed with saturated aqueous NH\(_4\)Cl (30 mL), dried over Na\(_2\)SO\(_4\), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (2 g, hexane/EtOAc = 8:2) to give the N-Boc pyrrolidine \(6c'\) (103.3 mg, 86%) as a colorless solid: IR (film) 2976, 1746, 1702, 1395, 1367, 1171, 681 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\), ca 1:1 mixture of rotamers) \(\delta\) 5.78 (d, \(J = 9.0\) Hz, 1 H), 5.62 (dd, \(J = 9.0, 2.0\) Hz, 1 H), 4.87 (br s, 1 H), 4.58 (br s, 1 H), 4.48 (s, 0.4 H), 4.40 (s, 0.6 H), 4.20 (br d, \(J = 19.0\) Hz, 1 H), 3.93 (d, \(J = 11.0\) Hz, 0.6 H), 3.84–3.82 (m, 0.4 H), 3.69 (s, 3 H), 3.67–3.58 (m, 1 H), 3.60 (s, 3 H), 3.55 (br d, \(J = 19.0\) Hz, 1 H), 2.96–2.91 (m, 1 H), 2.83–2.65 (m, 2 H), 1.5–1.38 (m, 18 H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\), selected) \(\delta\) 170.6, 169.7, 154.3, 153.6, 125.6, 125.3, 91.2, 80.4, 72.5, 70.5, 56.8, 52.1, 52.0, 51.8, 49.3, 47.6, 46.3, 40.5, 28.2 (\(\times\) 3), 28.1 (\(\times\) 3): HRMS (ESI, positive) calcd for C\(_{24}\)H\(_{36}\)N\(_2\)O\(_9\)Na [(M+Na)^+] 519.2313, found 519.2294.

4.1.16. General procedures for the synthesis of the glutamate analogs 7a–7d (as well as 1a–1d and 5a–5d).

A suspension of fully protected glutamate analogs 6a–6d in hydrochloric acid (6 M, 0.5 mL) was heated at 65 °C for 10 h. The reaction mixture was then cooled to rt and concentrated under reduced pressure. The residue was purified by column chromatography on reversed-phase silica gel (500 mg, water). The active fractions were lyophilized to afford the glutamate analogs 7a–7d.

4.1.17.
(3S,3aS,4aR,8aR,8bR)-3a-Carboxymethyl-8-oxa-2,3,3a,4a,7,8,8a,8b-octahydro-1H-benzofuro[2,3-c]pyrrole-3-carboxylic acid (7a).

With the general procedure above, 6a (13.6 mg, 0.034 mmol) was deprotected to give the glutamate analog 7a (8.2 mg, 79%) as a white solid: IR (film) 1713, 1634, 1402, 1029 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 6.11 (dd, J = 10.0, 3.5 Hz, 1 H), 5.91 (dd, J = 10.0, 3.5, 2.0 Hz, 1 H), 4.49 (s, 1 H), 4.43 (t, J = 1.5 Hz, 1 H), 4.11 (dd, J = 17.3, 3.5 Hz, 1 H), 4.05 (d, J = 2.5 Hz, 1 H), 4.02 (d, J = 17.3 Hz, 1 H), 3.93 (dd, J = 12.5, 10.0 Hz, 1 H), 3.20 (d, J = 17.0 Hz, 1 H), 3.15 (dd, J = 12.5, 8.5 Hz, 1 H), 3.10 (t, J = 8.5 Hz, 1 H), 2.88 (t, J = 17.0 Hz, 1 H); ¹³C NMR (125 MHz, D₂O/CD₃OD = 15:1) δ 174.0, 168.6, 132.4, 120.4, 90.6, 79.1, 72.8, 67.3, 64.3, 52.2, 45.7, 40.5; HRMS (ESI, positive) calcd for C₁₂H₁₆NO₆ [(M+H)+] 270.0978, found 270.0976.

4.1.18.
(Z,1S,3aR,3bS,8aS,9aS)-9a-Carboxymethyl-4,9-dioxa-2,3,3a,3b,4,5,6,8a,9,9a-decahydro-1H-azuleno[2,1-c]pyrrole-1-carboxylic acid (7b).

With the general procedure above, 6b (9.4 mg, 0.023 mmol) was deprotected to give the glutamate analog 7b (7.4 mg, 100%) as a white solid: IR (film) 1715, 1621, 1405, 1361, 1075 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 5.88 (ddd, J = 11.5, 5.5, 5.5 Hz, 1 H), 5.60 (dd, J = 4.0, 1.5 Hz, 1 H), 4.75 (s, 1 H), 4.16 (s, 1 H), 4.14 (d, J = 3.0 Hz, 1 H), 3.85 (dd, J = 17.5 Hz, 1 H), 3.84 (m, 1 H), 3.57 (m, 1 H), 3.15 (d, J = 17.5 Hz, 1 H), 3.12–3.02 (m, 2 H), 2.81 (d, J = 17.5 Hz, 1 H), 2.37–2.21 (m, 2 H); ¹³C NMR (125 MHz, D₂O/CD₃OD = 15:1) δ 174.6, 170.0, 132.0, 124.8, 88.8, 83.7, 81.0, 69.1, 67.6, 53.6, 46.2, 40.7, 29.7; HRMS (ESI, positive) calcd for C₁₃H₁₈NO₆ [(M+H)+] 284.1129, found 284.1128.

4.1.19.
(3S,3aS,4aR,8bS)-8-Aza-3a-carboxymethyl-2,3,3a,4a,7,8,8a,8b-octahydro-1H-benzofuro[2,3-c]pyrrole-3-carboxylic acid (7c).

With the general procedure above, 6c' (23.7 mg, 0.048 mmol) was deprotected to give the glutamate analog 7c (14.7 mg, 90%) as a white solid: IR (film) 1713, 1624, 1417, 1257, 1085, 967 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 6.11 (dd, J = 10.3, 4.5 Hz, 1 H), 6.07 (br d, 10.3 Hz, 1 H), 4.75 (br s, 1 H), 4.34 (s, 1 H), 3.99 (dd, J = 12.5, 9.0 Hz, 1 H), 3.86 (d, J = 4.0 Hz, 1 H), 3.78 (dd, J = 17.3, 4.0 Hz, 1 H), 3.65 (d, J = 17.3 Hz, 1 H), 3.48 (t, J = 9.0 Hz, 1 H), 3.32 (dd, J = 12.5, 9.0 Hz, 1 H), 3.08 (br s, 2 H); ¹³C NMR (125 MHz, D₂O/CD₃OD =
15:1 δ 174.2, 168.4, 126.7, 121.7, 89.3, 70.2, 65.5, 60.3, 49.1, 46.2, 42.3, 38.3; HRMS (ESI, positive) calcd for C_{12}H_{17}N_{2}O_{5} [(M+H)+] 269.1131, found 269.1130.

4.1.20.

(Z,1S*,3aR*,3bS*,8aS*,9aS*)-4-Aza-9a-carboxymethyl-2,3,3a,3b,4,5,6,8a,9,9a-decahydro-9-oxa-1H-azuleno[2,1-c]pyrrole-1-carboxylic acid (7d).

With the general procedure above, 6d (31.4 mg, 0.062 mmol) was deprotected to give the glutamate analog 7d (20.1 mg, 91%) as a white solid: IR (film) 1730, 1624, 1405, 1243, 1087, 991 cm\(^{-1}\); ¹H NMR (500 MHz, D\(_2\)O) δ 5.92 (m, 1 H), 5.69 (d, J = 11.0 HZ, 1 H), 5.13 (br s, 1 H), 4.15 (s, 1 H), 4.11 (d, J = 4.5 Hz, 1 H), 3.97 (dd, J = 13.0, 10.5 Hz, 1 H), 3.51 (t, J = 8.5 Hz, 1 H), 3.31–3.25 (m, 3 H), 3.09 (d, J = 18.0 Hz, 1 H), 2.92 (d, J = 18.0 Hz, 1 H), 2.55 (m, 1 H), 2.27 (m, 1 H); ¹³C NMR (125 MHz, D\(_2\)O/CD\(_3\)OD = 15:1) δ 173.3, 167.9, 127.7, 125.8, 87.9, 78.1, 65.2, 61.9, 51.1, 46.8, 45.4, 38.6, 21.4; HRMS (ESI, positive) calcd for C\(_{13}\)H\(_{19}\)N\(_2\)O\(_5\) [(M+H)+] 283.1288, found 283.1296.

4.1.21. Methyl (3S*,3aS*,4aR*,8aR*,8bS*)-8-aza-2,8-bis(tert-butoxycarbonyl)-3a-((methoxycarbonyl)methyl)-decahydro-2-methyl-1Hbenzofuro[2,3-c]pyrrole-3-carboxylate (16c').

To a stirred solution of 6c' (32.8 mg, 0.066 mmol) in methanol (6.0 mL) at rt was added palladium (10 wt% on carbon, 3.3 mg). The mixture was stirred vigorously under hydrogen atmosphere (1 atm) for 1 h. The catalyst was then removed by filtration and the filtrate was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (0.5 g, hexane/EtOAc = 8:2) to give 16c' (32.8 mg, 100%) as a white solid: IR (film) 1747, 1696, 1393, 1367, 1254, 1063, 770 cm\(^{-1}\); ¹H NMR (500 MHz, CDCl\(_3\), mixture of rotamers) δ 4.49–4.45 (m, 1 H), 4.28–4.21 (m, 2 H), 3.83–3.75 (m, 1 H), 3.73–3.69 (m, 4 H), 3.67–3.62 (m, 3 H), 3.55–3.50 (m, 1 H), 3.29–3.11 (m, 2 H), 2.76–2.64 (m, 2 H), 1.68–1.37 (m, 22 H); ¹³C NMR (125 MHz, CDCl\(_3\), selected) δ 171.2, 170.2, 156.0, 153.9, 90.9, 80.8, 74.9, 70.3, 61.0, 60.3, 52.2, 50.3, 49.3, 41.0, 39.8, 28.6 (× 3), 28.4 (× 3), 26.1, 19.4; HRMS (ESI, positive) calcd for C\(_{24}\)H\(_{38}\)N\(_2\)O\(_9\)Na [(M+Na)+] 521.2469, found 521.2466.

With the same procedure for the synthesis of 16c’, 16d (34.8 mg, 100%) was obtained as a white solid starting from 6d (34.7 mg, 0.069 mmol) and palladium (10 wt% on carbon, 3.5 mg).

Data for 16d. IR (film) 1747, 1692, 1393, 1209, 1016, 733 cm⁻¹; ¹H NMR (500 MHz, CDCl₃, ca 6:4 mixture of rotamers) δ 5.05 (m, 0.4 H), 4.45–4.40 (m, 1.6 H), 4.33–4.38 (m, 1 H), 3.89 (m, 0.6 H), 3.81–3.74 (m, 3.4 H), 3.67–3.62 (m, 4 H), 3.19–3.01 (m, 1.4 H), 2.88–2.73 (m, 2.6 H), 2.23 (m, 1 H), 1.85–1.38 (m, 14 H); ¹³C NMR (125 MHz, CDCl₃, selected) δ 153.9, 153.4, 117.7, 90.5, 81.1, 78.3, 67.7, 66.5, 53.3, 52.1, 52.0, 44.7, 37.6, 30.8, 28.2 (× 3), 28.1, 27.4, 20.9; HRMS (ESI, positive) calcd for C₂₂H₃₁N₂O₈F₃Na [(M+Na)+] 531.1925, found 531.1936.

4.1.23. (3S*,3aS*,4aR*,8aR*,8bS*)-8-Aza-3a-carboxymethyl-decahydro-1H-benzo[furazan[2,3-c]pyrrole-3-carboxylic acid (5c).

With the general procedure shown above, 16c’ (18.1 mg, 0.036 mmol) was deprotected to give the glutamate analog 5c (12.3 mg, 100%) as a white solid: IR (film) 1715, 1625, 1404, 1255, 975 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.47 (br s, 1 H), 4.25 (s, 1 H), 3.90 (t, J = 11.5 Hz, 1 H), 3.91 (s, 1 H), 3.37 (d, J = 12.5 Hz, 1 H), 3.32 (t, J = 9.0 Hz, 1 H), 3.22 (s, 1 H), 3.15 (d, J = 18.0 Hz, 1 H), 3.02 (d, J = 18.0 Hz, 1 H); ¹³C NMR (125 MHz, D₂O/CD₃OD = 15:1) δ 154.7, 168.4, 88.7, 72.4, 65.5, 59.7, 49.4, 45.6, 43.4, 37.2, 22.7, 16.5; HRMS (ESI, positive) calcd for C₁₂H₁₉N₂O₅ [(M+H)+] 271.1288, found 271.1291.


With the general procedure shown above, 16d (23.5 mg, 0.046 mmol) was deprotected to give the glutamate analog 5d (16.2 mg, 98%) as a white solid: IR (film) 1731, 1624, 1417, 1258, 1084 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.59 (dt, J = 7.5, 6.0 Hz, 1 H), 4.33 (s, 1 H), 3.97 (dd, J = 12.8, 10.0 Hz, 1 H), 3.86 (d, J = 4.5 Hz, 1 H), 3.44 (br s, 1 H), 3.43 (t, J = 9.0 Hz, 1 H), 3.28 (dd, J = 12.5, 9.0 Hz, 1 H), 3.08 (d, J = 18.0 Hz, 1 H), 2.99 (d, J = 18.0 Hz, 1 H), 2.94 (dd, J = 13.5, 3.5, 3.5 Hz, 1 H), 2.26 (m, 1 H), 1.88–1.60 (m, 4 H), 1.40 (dd, J =
13.5, 12.3 Hz, 1 H); \( ^{13} \text{C} \) NMR (125 MHz, D\(_2\)O/CD\(_3\)OD = 15:1) \( \delta \) 173.5, 168.0, 89.1, 78.4, 67.9, 65.3, 51.1, 49.4, 47.0, 38.6, 28.3, 26.4, 19.8; HRMS (ESI, positive) calcd for C\(_{13}\)H\(_{21}\)N\(_2\)O\(_5\) [(M+H)\(^+\)] 284.1445, found 284.1449.

4.1.25. Methyl \((3S,3aS,4aS,5S,6S,8aR,8bS)\)
2-(\textit{tert}-butoxycarbonyl)-3a-((methoxycarbonyl)methyl)-decahydro-5,6-dihydroxy-8-oxa-1\( H\)-benzofuro[2,3-c]pyrrole-3-carboxylate (17a).

To a stirred solution of 6a (5.40 mg, 0.0136 mmol) in \textit{tert}-butanol (0.2 mL) at rt was added a solution of NMO (100 mg, 0.85 mmol) in water (0.2 mL) and OsO\(_4\) (3.9 mM in \textit{tert}-butanol, 33 \( \mu \)L, 0.0014 mmol). After 3 h, saturated aqueous Na\(_2\)S\(_2\)O\(_4\) (2 mL) was added, and the mixture was extracted with chloroform (3 \( \times \) 5 mL). The combined extracts were washed with brine (2 mL), dried over Na\(_2\)SO\(_4\), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (0.5 g, methanol/chloroform = 2:98) to give the diol 17a (5.9 mg, 100%) as a white solid: IR (film) 3400, 1743, 1693, 1401, 1250, 1167, 1090 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of rotamers) \( \delta \) 4.71 (m, 1 H), 4.16–4.10 (m, 2 H), 4.01 (br s, 1 H), 3.92 (br s, 1 H), 3.81 (m, 1 H), 3.70–3.56 (m, 6 H), 3.44 (m, 1 H), 3.34–3.27 (m, 1 H), 3.08 (m, 1 H), 2.97–2.80 (m, 2 H), 2.64 (m, 1 H), 1.42–1.38 (m, 9 H); \(^{13} \text{C} \) NMR (125 MHz, CDCl\(_3\), selected) \( \delta \) 170.8, 170.3, 154.1, 91.9, 81.6, 80.8, 79.2, 69.3, 66.3, 64.4, 64.0, 52.1, 51.7, 50.9, 48.6, 40.2, 28.2 (\( \times \) 3); HRMS (ESI, positive) calcd for C\(_{19}\)H\(_{29}\)NO\(_{10}\)Na [(M+Na)\(^+\)] 454.1684, found 454.1678.

4.1.26. Methyl \((1S,3aR,3bR,7S,8R,8aR,9aS)\)
2-((\textit{tert}-butoxycarbonyl)-9a-((methoxycarbonyl)methyl)-dodecahydro-7,8-dihydroxy-4,9-dioxa-4-trifluoroacetyl-1\( H\)-azuleno[2,1-c]pyrrole-1-carboxylate (17b).

With the same procedure for the synthesis of 17a, 17b (2.9 mg, 100%) was obtained as a white solid starting from 6b (2.7 mg, 6.57 \( \mu \)mol).

Data for 17b. IR (film) 3406, 1742, 1694, 1394, 1171, 1101, 1074, 904, 756 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\), mixture of rotamers) \( \delta \) 4.62 (br s, 1 H), 4.38 (m, 1 H), 4.19 (br s, 1 H), 4.13–4.09 (m, 1 H), 3.91–3.84 (m, 2 H), 3.68–3.64 (m, 6 H), 3.32–3.24 (m, 1 H), 3.08–3.03 (m, 1 H), 2.98–2.90 (m, 2 H), 2.60–2.51 (m, 2 H), 1.92–1.86 (m, 1 H), 1.80–1.76 (m, 1 H), 1.42–1.38 (m, 9 H); \(^{13} \text{C} \) NMR (125 MHz, CDCl\(_3\), selected) \( \delta \) 171.1, 170.2, 153.8, 91.2, 85.5, 86.0, 80.6, 77.2, 71.8, 68.6, 67.7, 52.9, 52.1, 51.8, 49.1, 39.3, 34.7, 28.2 (\( \times \) 3); HRMS (ESI,
positive) calcd for C20H31NO10Na [(M+Na)\(^+\)] 468.1840, found 468.1840.

4.1.27. Methyl (3\(^S\),3a\(^S\),4a\(^S\),5\(^S\),6\(^S\),8a\(^R\),8b\(^S\))
8-aza-2,8-bis(tert-butoxycarbonyl)-3a-((methoxycarbonyl)methyl)-decahydro-5,6-dihydroxy-2-methyl-1H-benzofuro[2,3-c]pyrrole-3-carboxylate (17c).

With the same procedure for the synthesis of 17a, 17c (34.5 mg, 100\%) was obtained as a white solid starting from 6c (32.0 mg, 0.065 mmol).

Data for 17c. IR (film) 3412, 1744, 1698, 1396, 1367, 1170, 1133, 1057, 755 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\), ca 7:3 mixture of rotamers) \(\delta\) 4.70–4.62 (m, 1 H), 4.48 (br s, 0.3 H), 4.39 (br s, 0.7 H), 4.24 (m, 1 H), 3.96 (br s, 1 H), 3.89–3.81 (m, 1 H), 3.73 (br s, 3 H), 3.67–3.56 (m, 4 H), 3.23 (m, 1 H), 3.07–2.99 (m, 1 H), 2.68–2.65 (m, 2 H), 2.55–2.50 (m, 1 H), 2.26 (br s, 1 H), 1.47–1.44 (m, 18 H) \(\times\) 3); \(^1^3\)C NMR (125 MHz, CDCl\(_3\), selected) \(\delta\) 170.8, 170.2, 155.7, 153.5, 90.6, 81.4, 80.7, 79.0, 70.5, 66.4, 57.7, 52.3, 48.8, 47.6, 46.8, 43.2, 38.8, 28.2 (\(\times\) 3), 28.1 (\(\times\) 3); HRMS (ESI, positive) calcd for C\(_{24}\)H\(_{38}\)N\(_2\)O\(_{11}\)Na \([(M+Na)^{+}\)] 553.2368, found 553.2366.

4.1.28. Methyl (1\(^S\),3a\(^R\),3b\(^R\),7\(^S\),8\(^R\),8a\(^R\),9a\(^S\))

With the same procedure for the synthesis of 17a, 17d (35.1 mg, 100\%) was obtained as a white solid starting from 6d (33.1 mg, 0.065 mmol).

Data for 17d. IR (film) 3413, 1744, 1690, 1395, 1210, 1144, 1049, 756 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\), ca 6:4 mixture of rotamers) \(\delta\) 5.28 (s, 0.6 H), 5.07 (m, 0.4 H), 4.49–4.31 (m, 3.5 H), 4.00–3.89 (m, 2.5 H), 3.75–3.61 (m, 7 H), 3.29–2.77 (m, 4 H), 2.35–1.80 (m, 4 H), 1.45 (br s, 5.4 H), 1.39 (br s, 3.6 H) \(\times\) 3); \(^1^3\)C NMR (125 MHz, CDCl\(_3\), selected) \(\delta\) 170.5, 170.0, 154.2, 153.6, 115.6, 91.3, 81.7, 78.0, 73.4, 69.7, 68.0, 67.0, 52.4, 52.3, 50.3, 39.6, 37.9, 29.3, 28.4, 28.3 (\(\times\) 3); HRMS (ESI, positive) calcd for C\(_{22}\)H\(_{33}\)N\(_2\)O\(_{10}\)F\(_3\)Na [(M+Na)\(^{+}\)] 563.1823, found 563.1827.

4.1.29. (3\(^S\),3a\(^S\),4a\(^S\),5\(^S\),6\(^S\),8a\(^R\),8b\(^S\))-8-Aza-3a-carboxymethyl-decahydro-5,6-dihydroxy-1H-benzofuro[2,3-c]pyrrole-3-carboxylic acid (1c).
With the general procedure shown above, 17c (20.3 mg, 0.038 mmol) was deprotected to give the glutamate analog 1c (13.9 mg, 97%) as a white solid: IR (film) 3300, 1714, 1627, 1404, 1256, 1101, 1000 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.44 (br s, 1 H), 4.25 (s, 1 H), 4.18 (br s, 1 H), 3.98 (dd, J = 7.8, 3.5 Hz, 1 H), 3.89 (dd, J = 12.8, 10.0 Hz, 1 H), 3.85 (d, J = 2.5 Hz, 1 H), 3.36 (t, J = 8.5 Hz, 1 H), 3.24–3.12 (m, 3 H), 3.22 (t, J = 13.5 Hz, 1 H), 2.99 (d, J = 17.5 Hz, 1 H); ¹³C NMR (125 MHz, D₂O/CD₃OD = 15:1) δ 174.7, 168.4, 89.1, 77.8, 65.4, 64.9, 62.8, 57.4, 48.3, 45.6, 41.2, 37.1; HRMS (ESI, positive) calcd for C₁₂H₁₉N₂O₇ [(M+H)+] 303.1187, found 303.1190.

4.1.30.

With the general procedure above, 17d (23.3 mg, 0.043 mmol) was deprotected to give the glutamate analog 1d (16.7 mg, 100%) as a white solid: IR (film) 3350, 1718, 1635, 1405, 1227, 1097, 991 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.37 (t, J = 5.5 Hz, 1 H), 4.31 (s, 1 H), 4.13 (br s, 1 H), 3.98 (dd, J = 13.0, 10.0 Hz, 1 H), 3.96 (d, J = 5.5 Hz, 1 H), 3.92 (d, J = 5.5 Hz, 1 H), 3.46 (t, J = 8.5 Hz, 1 H), 3.31 (d, J = 12.5 Hz, 1 H), 3.31 (t, J = 13.0 Hz, 1 H), 3.16 (m, 1 H), 3.11 (d, J = 18.0 Hz, 1 H), 3.00 (d, J = 18.0 Hz, 1 H), 1.98–1.97 (m, 2 H); ¹³C NMR (125 MHz, D₂O/CD₃OD = 15:1) δ 173.5, 168.0, 88.8, 81.3, 74.8, 71.3, 65.5, 65.2, 51.1, 47.0, 44.0, 38.7, 29.5; HRMS (ESI, positive) calcd for C₁₃H₂₁N₂O₇ [(M+H)+] 317.1343, found 317.1352.

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References and notes

19. (N,N-Dialkyl)amides were reported to be used in Kuwano's method, whereas (N-monoalkyl)amides are used for reaction with Meerwein reagent.
21. The stereoselectivity for dihydroxylation of 6c' and 6d was unambiguously determined as follows. Unfortunately, 17c' and 17d were obtained as a mixture of rotamers. However, after deprotection, 1c and 1d gave clear 1H NMR spectra, which strongly support the stereochemistries shown in Scheme 9. In addition, JH,H values of 1c and 1d were in good accord with those of 1a and 1b, whose stereostructures had been established previously. For details, see Supporting Information.
23. All the animals were maintained according to the National Research Council's Guide for the Care and Use of Laboratory Animals.
24. Generally, ligands for glutamate receptors (GluR) induce dose-dependent behavioral toxicity, see reference 10.
Legends for figures and schemes

Figure 1. Dysiherbaine congeners,\textsuperscript{5,6} antagonistic analog MSVIII\textsuperscript{-19},\textsuperscript{9} and hypoactive artificial glutamate analog 1a,\textsuperscript{11}

Scheme 1. Our first-generation synthetic pathway toward artificial glutamate analogs.\textsuperscript{11}

Scheme 2. The second-generation synthetic plan for artificial glutamate analogs (in the present study).

Scheme 3. Synthesis of common intermediates 4c and 4d.

Scheme 4. Synthesis of advanced intermediates 6a–6d by an improved deoxygenation.

Scheme 5. An attempt to deoxygenate pyrrolidone lactam 11 by rhodium-catalyzed hydrosilylation.

Scheme 6. Undesired decomposition of N–Ac group under Meerwein conditions.

Scheme 7. Synthesis of glutamate analogs 7a–7d with unsaturation at the C ring.

Scheme 8. Synthesis of glutamate analogs 5a–5d with saturation at the C ring.

Scheme 9. Synthesis of dihydroxylated glutamate analogs 1a–1d.
dysiherbaine (R = –NHMe)
neodysiherbaine A (R = –OH)

Figure 1

MSVIII-19

1a
Scheme 1

2a (n = 1), 2b (n = 2) → domino metathesis (ROM/CM/RCM) → PMB

Glu unit

4 steps → common intermediates

3a (n = 1): 100%
3b (n = 2): 84%

1-1) hydrogenation
1-2) deoxygenation of lactam (low yield)

1-3) protective group manipulation (PMB → Boc)
1-4) global deprotection

5a (n = 1): 25%, hyperactive
5b (n = 2): 43%, hyperactive

2-1) dihydroxylation
2-2) protection
2-3) same sequence of reactions as above (1-2 → 1-4)

4a (n = 1): 76%
4b (n = 2): 58%

1a (n = 1): 53%, hypoactive
1b (n = 2): 27%, hyperactive
Scheme 2

1) protective group manipulation
2) deoxygenation of lactam

common intermediates

\[ X = \begin{align*}
-\text{O—} & (4a) \\
-\text{OCH}_2 & (4b) \\
-N(\text{Ns}) & (4c) \\
-N(\text{TFA})\text{CH}_2 & (4d)
\end{align*} \]

advanced intermediates

\[ 6a-6d \]

2 steps

\[ 1a-1d \]
dihydroxylated glutamate analogs

\[ 5a-5d \]
saturated glutamate analogs

1 step

\[ 7a-7d \]
unsaturated glutamate analogs
Scheme 3

3c (X = \text{N(Ns)})
3d (X = \text{N(TFA)CH}_2)

8c: 90%
8d: 83%

9c: 81%
9d: 91%

common intermediates in G1 synthesis
Scheme 4

common intermediates in G1 synthesis

4a (X = -O–) 10a: 71%
4b (X = -OCH$_2$–) 10b: 71%
4c (X = -N(Ns)–) 10c: 78%
4d (X = -N(TFA)CH$_2$–) 10d: 80%

1) Me$_3$O·BF$_4$
K$_2$CO$_3$
CH$_2$Cl$_2$

2) NaBH$_3$CN
CF$_3$COOH
MeOH

3) Boc$_2$O, TEA
CH$_2$Cl$_2$

advanced intermediates

6a: 59%
6b: 85%
6c: 86%
6d: 84%

6c' (X = -N(Boc)–)

1) PhSH, Cs$_2$CO$_3$
CH$_3$CN
2) Boc$_2$O, TEA
CHCl$_3$
86%
Scheme 5

1) \( \text{Et}_2\text{SiH}_2 \), \( \text{RhH(CO)(PPh}_3)_3 \), rt; 1 M hydrochloric acid

2) \( \text{Boc}_2\text{O} \)

23%
Scheme 6

\[
\begin{align*}
\text{Me}_3\text{O} \cdot \text{BF}_4 & \quad \text{K}_2\text{CO}_3 \\
\rightarrow & \quad \text{CH}_2\text{Cl}_2 \\
\text{NaBH}_3\text{CN} & \quad \text{CF}_3\text{COOH} \\
\rightarrow & \quad \text{MeOH}
\end{align*}
\]

obtained as an inseparable mixture in 88% yield.
Scheme 7

6a, 6b, 6c', 6d → 7a (Y = –O–): 79%
7b (Y = –OCH₂–): 100%
7c (Y = –NH–): 90%
7d (Y = –NHCH₂–): 91%

advanced intermediates
Scheme 8

6a, 6b, 6c', 6d
advanced intermediates

\[ \text{H}_2, 10\% \text{ Pd/C} \rightarrow \text{MeOH} \]

16a (X = –O–): 100%\textsuperscript{11}
16b (X = –OCH}_2\text{–}): 96%\textsuperscript{11}
16c' (X = –N(Boc)–): 100%
16d (X = –N(TFA)CH}_2\text{–}): 100%

hydrochloric acid (6 M)

\[ 65 \, ^\circ \text{C} \]

5a (Y = –O–): 63%\textsuperscript{11}
5b (Y = –OCH}_2\text{–}): 77%\textsuperscript{11}
5c (Y = –NH–): 100%
5d (Y = –NHCH}_2\text{–}): 98%
Scheme 9

6a, 6b, 6c', 6d

advanced intermediates

OsO₄, NMO

tBuOH, H₂O

17a (X = -O-): 100%
17b (X = -OCH₂-): 100%
17c' (X = -N(Boc)-): 100%
17d (X = -N(TFA)CH₂-): 100%

hydrochloric acid (6 M)

65 °C

1a (Y = -O-): 74%¹¹
1b (Y = -OCH₂-): 100%¹¹
1c (Y = -NH-): 97%
1d (Y = -NHCH₂-): 100%