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Thiol addition to *t*-butyl methyl squarate. Efficient synthesis of novel sulfur-linked squaryl group-containing glutamate analogs

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ABSTRACT

Novel sulfur-linked squaryl group-containing glutamate analogs were synthesized via addition and mono-substitution reactions of thiols to *t*-butyl methyl squarate (BMSQ: **7**) in two steps. A glutamate analog prepared from Boc-L-Cys showed a potent binding affinity to KA/AMPA receptors.

Squaric acid is known as an oxocarbon possessing an aromaticity, strong acidity, hydrogen bonding, and metal chelating properties.[1] These physicochemical properties have attracted significant attention in medicinal chemistry as an equivalent functional group of a carboxylic acid.[2] The substitution of the carboxyl group in L-glutamic acid by other anionic (proton-donating) functional groups, such as the isoxazole or phosphonate group, has led to the development of novel glutamate analogs which are used as essential tools to investigate neurobiological functions of glutamate receptors in mammalian CNS, that is, L-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) exhibits a potent and selective activity as a representative agonist of AMPA receptors among the ionotropic glutamate receptors [iGluRs which are divided into *N*-methyl-D-aspartic acid (NMDA), AMPA, and kainic acid (KA) subtypes], and L-2-amino-4-phosphonobutyric acid (L-AP4) is used as a selective agonist of group III metabotropic glutamate receptors (mGluRs which are classified into group I–III subtypes).[3] Recently, we and other groups reported the synthesis of the squaryl group-containing glutamate analogs **1–3**,[4–6] in which the squaryl (Sq) group was incorporated into L-glutamate instead of one of its carboxyl groups. The carbon- and nitrogen-connected squaryl group-containing glutamate analogs, C–Sq–Glu **1**[4] and N–Sq–Glu **2**[5], exhibited a potent binding activity to iGluRs, in particular, AMPA and KA receptors. In this report, we describe the synthesis of novel sulfur-linked Sq group-containing glutamate analogs (S–Sq–Glu) **4–6** via the addition of a thiol group-containing α-amino acid to *t*-butyl methyl squarate (BMSQ) (**7**) (Fig. 1). Their binding activities to iGluRs are briefly disclosed.

According to our previous studies, individual GluRs recognize a specific conformation of L-glutamate (conformational requirement of glutamate receptors).[7] Since the sulfur-linked Sq group-containing glutamate analog, such as **4**, would have a slightly different conformational and/or stereoelectronic properties in comparison with those of the carbon- or nitrogen-linked analogs, **1** and **2**, in view of its longer C–S and S–Sq bond lengths, it was of particular interest to examine their neuropharmacological activity toward glutamate receptors. In addition to **4**, its β,β-dimethyl analog **5**, one carbon-elongated analog **6**, and their enantiomers were chosen as the present synthetic targets which would provide useful information for the SAR studies of the glutamate analogs. We considered that the S–Sq–Glu analogs could be simply synthesized by the addition–substitution reaction of a dialkyl squarate with the terminal thiol group of readily available L-cystein (Cys), D- and L-penicillamine, and D- and L-homocystein (hCys). Prior to their syntheses, the stability of the RS–Sq (R = alkyl) group under physiological conditions had to be estimated because the RS–Sq group can be viewed as an activated ester group. To answer this question, Bellus reported that the CH_3S–Sq bond is quite resistant to acid hydrolysis upon heating at 100 °C in 13% HCl for two days, while the CH_3O–Sq group was readily hydrolyzed under acidic or basic conditions to give squaric acid.[8] Several methods have been reported for the synthesis of the alkyl- or arylthioesters of squaric acid: (i) [2+2] cycloaddition of methylthioketene,[8] (ii) addition of hydrogen sulfide to diethyl squarate followed by S-alkylation,[9] (iii) addition–substitution of alkyl thiols with 1,2-dichlorocyclobutene-3,4-dione,[9] and (iv) addition–substitution of alkyl thiols with dimethyl squarate.[10] These methods except for (i) gave 1,2-disubstituted thioesters as an exclusive or major product. These important studies suggested that the development of an efficient method for the mono-substitution reaction of the squarate with a thiol is necessary prior to the synthesis of **4–6**. We envisioned that the thiol addition to *t*-butyl methyl squarate (BMSQ) **7**[11] would undergo an addition–substitution reaction at the vinylic carbon attached to the methoxy group to give a mono-substituted adduct in which the sterically bulky *t*-butoxy group remained unsubstituted. The *t*-butyl group of the resulting product can be removed under mild acidic conditions to give the mono-substituted RS–Sq derivatives (Eq. 1).

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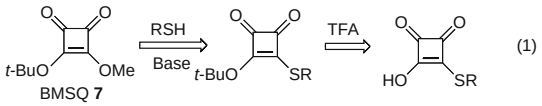


Initially, we examined the thiol addition to BMSQ using dodecane-1-thiol (**8a**) as a model compound. After screening of several bases (Et_3N, DBU, 1,5,7-triazabicyclo[4.4.0]dec-1-ene (TBD), or pH 8 buffer), it was found that triethylamine effected the desired reaction to give the mono-substituted adduct **9a** in 92% yield. None of the disubstituted product was observed. Other bases resulted in decreased yields due probably to instability of BMSQ to the strong bases. The *t*-butyl group of the resulting **9a** was simply removed by its exposure to trifluoroacetic acid (TFA) to give **10a** in quantitative yield. This method was applicable to a variety of alkyl thiols as shown in Table 1. The sterically bulky thiol **8b** reacted with **7** to give **9b** in 59% yield (entry 2). The ester and *N*-Boc groups were tolerated under the standard reaction conditions. The *t*-butyl groups of **9b–d** were removed by treatment with TFA to give the S–Sq derivatives **10b–d** in excellent yields (entries 2–4), respectively, in which **10d** can be viewed as a novel S–Sq analog of γ-aminobutyric acid (GABA). On the other hand, the reaction with the aromatic thiol gave the mono-substitution product **9e** in 12% yield and the methyl thioether **11** was isolated as the major product (48%) (entry 5) due to the attack of the aromatic thiolate, which is a soft nucleophile, to the methoxy carbon of **7**.[12]

With the above mentioned method in hand, we turned our attention to the synthesis of the S–Sq–Glu analogs **4-6**. The treatment of Boc–L-Cys **12** with **7** under the standard reaction conditions furnished the mono-substitution product **13**, which, upon treatment with TFA, afforded the sulfur-linked glutamate analog **4** in 72% overall yield; $[\alpha]_D^{27}$ − 60.4 (*c* 0.97, 6 N HCl). The structure was confirmed by the X-ray crystallographic analysis (Fig. 2),[13] in

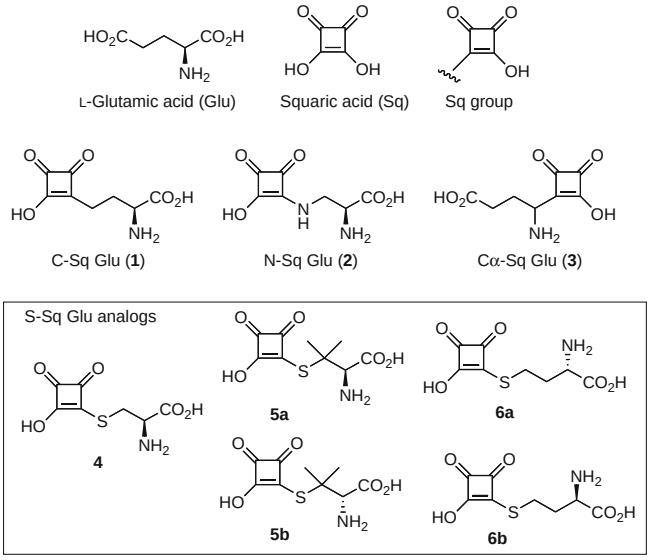


Figure 1.

which its solid conformation was similar to that of the reported N–Sq–Glu **2** (Na salt)[5] and one of the preferential conformers of L-glutamate in the crystal form.[14] The bond angle (H_2C–S–Sq) was 68.6°, and the H_2C–S and S–Sq bond lengths were 1.809 and 1.714 Å, respectively. The S–Sq bond length was much longer than the N–Sq (1.32 Å) and C–Sq (1.50 Å) bond lengths. The synthetic sulfur-linked analog **4** was quite stable even at pH 0.3 in D_2O for one week. The optical stability of **4** was ascertained by the fact that no D atom was incorporated into its α-carbon under the above conditions. On the other hand, the amino acid **4** gradually decomposed below pH 8 probably due to an attack of the internal α-amino

Table 1
Addition of thiols to BMSQ **7**

Entry	Thiol	Time	Product[a] (yield (%))	Product[b] (yield (%))
1	$HS(CH_2)_{11}CH_3$	4	**9a** (92)	**10a** (100)
2	**8b**	9	**9b** (59)	**10b** (84)
3	**8c**	9	**9c** (91)	**10c** (93)
4	**8d**	20	**9d** (92)	**10d** (92)
5	**8e**	24	**9e** (12)	**11** (48)

[a] All reactions with **7** were carried out in CH_2Cl_2 at room temperature in the presence of Et_3N (1 equiv).
[b] All reactions were carried out in CH_2Cl_2 and TFA at room temperature.

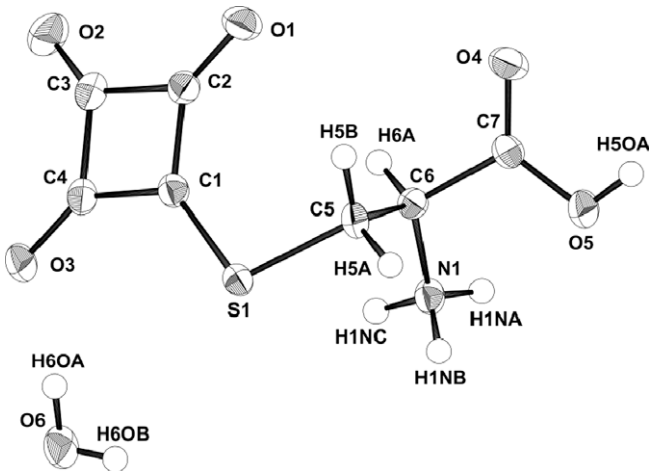


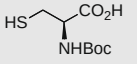
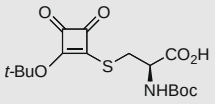
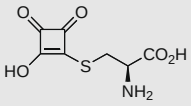
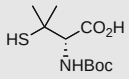
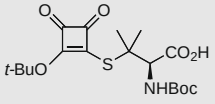
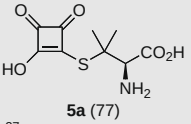
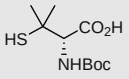
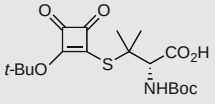
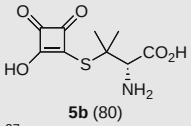
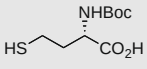
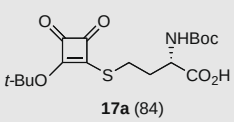
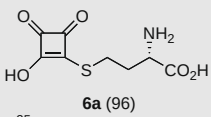
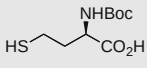
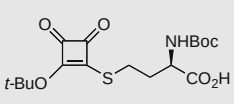
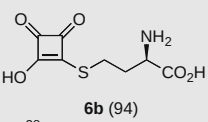
Figure 2. X-ray crystal structure of **4**.

group on the oxo- or thioester-attached carbon atom. The pK_1 (~0) and pK_2 (1.9) values were calculated by titration experiments using ^{1}H NMR (see, Supplementary data) although the pK_3 value could not be obtained due to the above mentioned reason. These values were almost the same as those of the carbon analog **1** (pK_1 ~0 and pK_2 1.8)[4] and were stronger than those of squaric acid (0.54 and 3.48).[1d] Finally, the other analogs **5a**,**b** and **6a**,**b** were synthesized from Boc–penicillamine **14a**,**b**, and Boc–hCys **16a**,**b** according to the same procedure as those for the synthesis of **4**, respectively (Table 2).

Preliminary pharmacological assays of the synthetic compounds **4**, **5a**,**b**, and **6a**,**b** for ionotropic glutamate receptors (KA, AMPA, and NMDA subtypes) in rat brain synaptic membranes were performed. Radioligand binding assays using [^{3}H]KA for the KA receptors, [^{3}H]AMPA for the AMPA receptors, and [^{3}H]CGP39653 for the NMDA receptors revealed that S–Sq–L-Cys **4** exhibited a potent and selective binding affinity to KA (IC_{50} = 3.8 μM) and AMPA receptors (1.4 μM),[15] which were almost the same in magnitude as those of the C–Sq analog **1** (0.8 μM for AMPA and 2 μM for KA),[4] while the magnitude of its activity was weaker than that of L-glutamic acid. In contrast, both enantiomers of the S–Sq–hCys **6a**,**b** were found to exhibit selective binding affinities to the NMDA receptors (**6a**, 1.0 μM and **6b**, 0.5 μM) rather than the other receptors (24 μM for AMPA and >100 μM for KA)). The penicillamine analogs **5a**,**b** did not show any significant binding affinity to these receptors even at a 100 μM concentration.

In summary, we have developed a facile method to access various sulfur-linked Sq derivatives including new glutamate analogs **4**, **5a**,**b**, and **6a**,**b** using the mono-substitution reaction of BMSQ **7**. Among the new analogs, the L-Cys-derived **4** exhibited a potent binding affinity to the AMPA/KA receptors and S–Sq–hCys **6a**,**b** showed intriguing binding affinities to the NMDA receptors. Further pharmacological characterization of these analogs to gluta-

Table 2
Synthesis of S–Sq group-containing glutamate analogs **4**, **5a**,**b**, and **6a**,**b**

Entry	Thiol	Time	Product[a] (yield (%))	Product[b] (yield (%))
1	 Boc-L-cysteine **12**	18	 **13** (77)	 **4** (93) $[\alpha]^{27}_D$ -60.4 (c 0.97, 6 *N* HCl)
2	 Boc-L-penicillamine **14a**	24	 **15a** (75)	 **5a** (77) $[\alpha]^{27}_D$ -20.7 (c 0.97, 6 *N* HCl)
3	 Boc-D-penicillamine **14b**	24	 **15b** (76)	 **5b** (80) $[\alpha]^{27}_D$ +23.3 (c 0.86, 6 *N* HCl)
4	 Boc-L-homocysteine **16a**	18	 **17a** (84)	 **6a** (96) $[\alpha]^{25}_D$ +30.8 (c 1.00, 1 *N* HCl).
5	 Boc-D-homocysteine **16b**	18	 **17b** (87)	 **6b** (94) $[\alpha]^{28}_D$ +29.5 (c 1.0, 1 *N* HCl)

[a] All reactions with **7** were carried out in CH_2Cl_2 at room temperature in the presence of Et_3N (2 equiv).
[b] All reactions were carried out in CH_2Cl_2 and TFA at room temperature.

mate receptors and their SAR studies of other glutamate analogs are ongoing in our laboratories.

Acknowledgments

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Supplementary data

Supplementary data (experimental details, characterization data, and titration graphs for determination of the p*K*a values of **4**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.05.037.

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11. (a) Liu, H.; Tomooka, C. S.; Moore, H. W. *Synth. Commun.* **1997**, *27*, 2177–2180; (b) Modified procedure for the preparation of BMSQ by us (39% yield from dimethyl squarate) is described in Supplementary data.
12. It has been reported that the reaction of dimethyl squarate with benzenethiol in THF gave a mixture of the mono-substituted product (35%) and 1,2-addition products (60%).[10] However, the use of 4-(trimethylsilyl)benzenethiol under the reported conditions resulted in almost the recovery of the starting dimethyl squarate, and a small amount of 4-(trimethylsilyl)benzene thiomethyl ether was isolated.
13. CCDC 721881 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Center via http://www.ccdc.cam.ac.uk/deposit
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15. Binding affinity of **4** to the NMDA receptors was >100 μM.