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Efficient Synthesis of a Novel 4-Hydroxy-2,3-dioxocyclobut-1-enyl Group Containing Amino Acids

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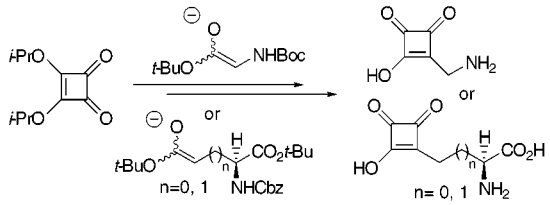
ABSTRACT



Syntheses of novel amino acids possessing a squaryl group, 1–3, in an optically active form are described. The syntheses of 1–3 were conducted by a concise route involving (1) an aldol addition of an enolate derived from Gly, L-Asp, or L-Glu to diisopropyl squarate (4) and (2) an efficient decarboxylation of the aldol adducts based on the electron-withdrawing property of the squaryl group. Introducing *N*-protected group to 2 and 3 and peptide formation reactions are also described.

Squaric acid is known as an oxocarbon possessing aromaticity and strong acidity.[1] These unique properties have been extended to the development of advanced materials[2] and employed as a useful diene synthon in organic synthesis.[3] Recently, in medicinal chemistry, the concept of the use of the 4-hydroxy-2,3-dioxocyclobut-1-enyl (squaryl) group as an isostere of carboxylic acid has arisen, in which this moiety was incorporated into the substructure of lactivicin,[4] phosphonoacetic acid,[5] glycine,[6] γ-aminobutyric acid,[7] *N*-methyl-D-aspartic acid,[7] and L-glutamate analogues.[8,9] The squaryl group of these compounds was mainly connected by a nitrogen or a phosphine atom with other functional groups

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although the strong acidity of the squaryl group would be much reduced. Only a few examples have been reported regarding the analogues which are connected by a carbon−carbon bond. These facts prompted us to examine the syntheses of amino acids containing a squaryl group, nearly equivalent to naturally occurring acidic amino acids such as aspartic acid, glutamic acid, and tyrosine, which would provide numerous applications to life science. In this report, we wish to describe the syntheses of 2-squarylmethylamine (**1**), (2*S*)-2-amino-3-squarylpropionic acid (**2**), and (2*S*)-2-amino-4-squarylbutyric acid (**3**) via a facile addition of enolates (Figure 1).

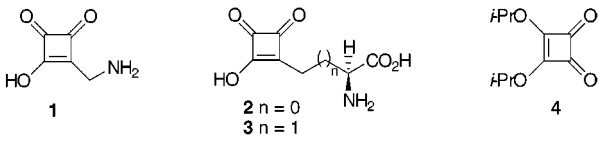
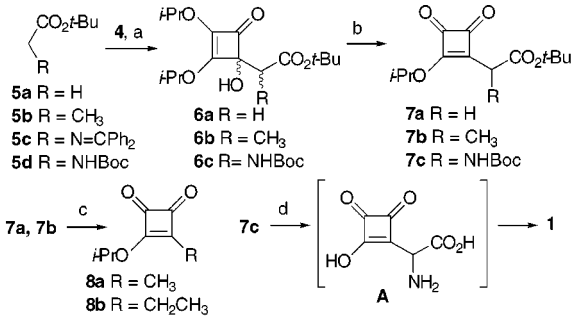


Figure 1. Squaryl group containing amino acids and diisopropyl squarate.

We began the studies with (1) an inspection of an appropriate protecting group for the dihydroxy groups of squaric acid suitable throughout the synthetic transformations and (2) finding an efficient method for introducing an optically active amino acid moiety where an addition of an ester enolate derived from the amino acid to a carbonyl group of the squarate was our choice (vide infra). Previous studies regarding the addition of enolates to dimethyl or diethyl squarate have shown that the yields of the corresponding 1,2-adducts are moderate due probably to simultaneous 1,4-addition of the reagent or the amine employed as the base to the squarate.[10] To avoid the presumed side reactions, we employed the squarate **4** protected with a sterically bulky isopropyl group. Then, the yield of the enolate addition using *tert*-butyl acetate (**5a)** was much improved to give **6a** in 98% yield. In the case of the enolate prepared from *tert*-butyl propionate (**5b)**, the reaction required an addition of ceric chloride for improved yield (**6b**, 71%). Treatment of the adducts with a few drops of 12 M HCl in CH_2Cl_2 afforded *tert*-butyl 2-squaryl acetates, **7a**[11] and **7b**, respectively (Scheme 1).

Since the squaryl group can be viewed as a potent electron-withdrawing group, we assumed that the carboxy group in

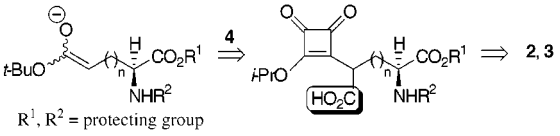
Scheme 1[a]



[a] (a) 1 equiv LDA, THF, -78 °C, 2 h, **6a** (98%); 1 equiv LDA, $CeCl_3$, THF, -78 °C, 2 h, **6b** (71%); 2 equiv LDA, THF, -78 °C, 0.5 h, **6c** (72%); (b) 12 M HCl, CH_2Cl_2, rt, 4 h, **7a** (93%), **7b** (76%), **7c** (84%); (c) (i) TFA, CH_2Cl_2, rt, 8 h; (ii) 2-3 equiv Et_3N, CH_2Cl_2, rt, 15 min, **8a** (2 steps, 59%), **8b** (2 steps, 57%); (d) 12 M HCl, acetone, rt, 0.5 h, **1** (72%).

7 would be removed under the decarboxylative conditions. In fact, successive treatments of the esters **7a** and **7b** with trifluoroacetic acid followed by refluxing in toluene gave in moderate yield the decarboxylated products, **8a**[12] and **8b**,[13] respectively. Instead of heating, treatment with triethylamine (2−3 equiv) in CH_2Cl_2 at room temperature for 15 min afforded the same products.[14] The isopropyl group was removed using 12 M HCl in acetone (1:1) to give 3-hydroxy-4-methylcyclobutene-1,2-dione[13] (98%) and 3-ethyl-4-hydroxycyclobutene-1,2-dione[15] (98%), respectively. Based on these results, it would be reasonable to plan the synthesis of **1**−**3** by the following strategy: (1) addition of an ester enolate derived from glycine, L-aspartic acid, or L-glutamic acid as the aldol donor to diisopropyl squarate (**4**), (2) decarboxylation of the resulting aldol adduct, and (3) deprotection leading to the amino acids **1**−**3** (Scheme 2).

Scheme 2



First, we attempted an aldol addition of various types of glycine enolate to **4**. The dianionic enolate **5d** underwent smooth addition at −78 °C to give aldol adduct **6c** in 72% yield as a mixture of diastereomers, while the reaction with the imino enolate **5c** did not proceed even at ambient

(9) For related examples, see: (a) Oguz, U.; Akkaya, E. U. *J. Org. Chem.* **1998**, *63*, 6059−6060. (b) Pirrung, M. C.; Han, H.; Chen, J. *J. Org. Chem.* **1996**, *61*, 4527−4531. (c) Kamath, V. P.; Diedrich, P.; Hindsgaul, O. *Glycoconjugate J.* **1996**, *13*, 315−319. (d) Soll, R. M.; Kenney, W. A.; Primeau, J.; Garrick, L.; McCaully, R. J.; Colatsky, T.; Oshiro, G.; Park, C. H.; Hartupee, D.; White, V.; McCallum. J.; Russo, A.; Dinish, J.; Wojdan, A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 757−760. (e) Young, R. C.; Durant, G. J.; Emmett, J. C.; Ganellin, R.; Graham, M. J.; Mitchell, R. C.; Prain, H. D.; Roantree, M. L. *J. Med. Chem.* **1986**, *29*, 44−49.

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(14) Removal of the alkoxycarbonyl group was also effected by treatment with LiI in DMF at 120 °C (47%).

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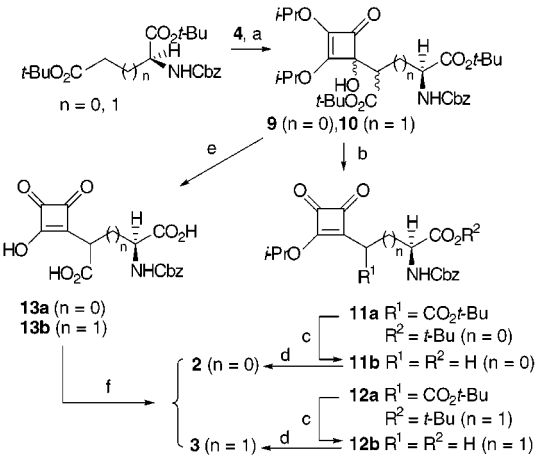
temperature.[16] Treatment of **6c** with a few drops of 12 M HCl gave protected 2-squarylglycine **7c** (84%), which, upon exposure to excess 12 M HCl in acetone at room temperature, underwent initial deprotection and subsequent decarboxylation to give **1**[6] (72%). This concomitant decarboxylation in contrast to the model study would be due to the presence of an internal amino group which enhances the rate of decarboxylation at room temperature.

Next, we attempted to introduce an optically active alanyl moiety into **4** (Scheme 3). Di-*tert*-butyl Cbz-L-aspartate was

Scheme 3[a]



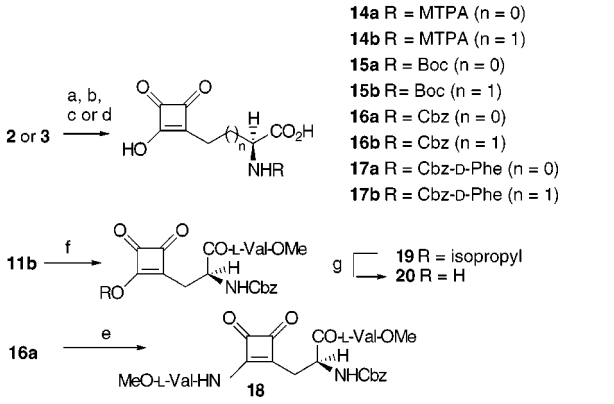
[a] (a) 2.2 equiv LHMDS, 6 equiv LiCl, DMPU, THF, -45 °C, 1 h, **9** (92%), **10** (92%); (b)12 M HCl, CH_2Cl_2, rt, 12 h, **11a** (63%), **12a** (90%); (c) (i) TFA, CH_2Cl_2, rt, 12 h; (ii) Et_3N, CH_2Cl_2, rt, 10 min, **11b** (2 steps, 80%), **12b** (2 steps, 78%); (d) 12 M HCl, acetone, **2** (87%), **3** (98%); (e) 12 M HCl, acetone, rt, 7 h; (f) 6 M HCl, reflux, 12 h, **2** (2 steps, 68%), **3** (2 steps, 74%)

chosen as the enolate precursor. After numerous unsuccessful trials regarding the choice of base, solvent, and additives,[17] we finally found that an addition of LiCl and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU), which dramatically increased the solubility of the resulting dianionic enolate in THF as reported by Seebach,[18] was quite effective, giving in 92% yield the desired aldol adduct **9** as a mixture of diastereomers. The mixture was converted into dicarboxylate **11a** with a few drops of 12 M HCl in CH_2Cl_2. Decarboxylation at C3 of **11a** was effected by triethylamine to give **11b** in the same manner as the conversion of **7** to **8**. Finally, all the protecting groups were removed, simultaneously, with 6 M HCl to afford the desired amino acid **2** in 87% yield. This process could be shortened substantially by subjecting the aldol adduct **9** to 12 M HCl in acetone at room temperature followed by refluxing in 6 M HCl to give **2** via **13a** (68%, **2**: mp 170−171 °C (dec); $[\alpha]_D$ +15.6° (*c* 1.0, 6 M HCl)). The synthesis of **3** was performed in essentially the same manner as that of the aspartate using di-*tert*-butyl Cbz-L-glutamate. The aldol adduct **10** was readily transformed into **13b**, which, upon heating with 6 M HCl, gave **3**: mp 160−163 °C (dec); $[\alpha]_D$ +38.9° (*c* 1.0, 6 M HCl). The optical purity of these amino acids was determined to be >90% ee by ^{1}H NMR analyses of the corresponding (*R*)-MTPA amides **14a** and **14b**. Thus, squaryl amino acids **2** and **3** were synthesized from **4** in a few steps in optically active form.

Both **2** and **3** are potent acidic compounds, and their pK_a values were ∼0, 1.5, 9.4 for **2** and ∼0, 1.8, 9.4 for **3**, respectively.[19] The smallest values are compatible with that of 3-hydroxy-4-methylcyclobutene-1,2-dione (0.24) and 3-hydroxy-4-phenylcyclobutene-1,2-dione (−0.22).[1b] Indeed, the inorganic salt-free 3-hydroxycyclobutene-1,2-diones **14**−**17** were not extracted with ethyl acetate from the 2 M HCl solution, but from 6 M HCl.[20]

Peptide couplings of the *N*-terminal of **2** or **3** with Cbz-D-Phe-OSu were performed under the biphasic conditions to give **17** (Scheme 4). On the other hand, coupling of

Scheme 4[a]



[a] (a) 3 equiv MTPACl, $NaHCO_3$, Et_2O, H_2O, rt, 12 h, then 6 M HCl, **14a** (80%), **14b** (98%); (b) 3 equiv Boc_2O, $NaHCO_3$, Et_2O, H_2O, rt, 12 h, then 6 M HCl, **15a** (15%), **15b** (27%); (c) 3 equiv CbzCl, $NaHCO_3$, Et_2O, H_2O, rt, 12 h, then 6 M HCl, **16a** (84%), **16b** (66%); (d) 3 equiv Cbz-D-Phe-OSu, $NaHCO_3$, AcOEt, H_2O, rt, 12 h, then 6 M HCl, **17a** (85%), **17b** (50%); (e) L-Val-OMe• HCl, EDCI, Et_3N, DMF, rt, 12 h, **18** (7%); (f) L-Val-OMe• HCl, EDCI, DMAP, CH_2Cl_2, rt, 12 h, **19** (40%); (g) 1 M HCl, acetone, rt, 4h, **20** (88%)

N-protected **16a** with L-Val-OMe afforded unexpected **18** having two valine moieties. To avoid the side reactions, the protected **11b** was used for the coupling to give the desired dipeptide **19**. This was converted to the desired **20** by the use of 1 M HCl in acetone. Thus, the modification of **2** and **3** at their *N*-terminal was achieved by the use of a Schotten-

(16) For dianionic glycine enolates, see: Evans, D. A.; Sidebottom, P. J. *J. Chem. Soc., Chem. Commun.* **1978**, 753−754.

(17) The reaction of **4** with a dienolate prepared from L-Asp using LDA or KHMDS in THF, toluene, or hexane afforded the aldol adducts in poor yield (∼10%). This was slightly improved (∼20%) when $CeCl_3$ was added.

(18) Seebach, D.; Bossler, H.; Grundler, H.; Shoda, S. *Helv. Chim. Acta* **1991**, *74*, 197−224.

(19) Supporting Information.

(20) When the *N*-Boc-protected compound was employed, the organic phase should immediately be washed with brine.

Bauman-type method, and the coupling at their *C*-terminal was performed using the protected intermediate **11b**.

In summary, we have synthesized three kinds of the amino acids, **1**, (2*S*)-**2**, and (2*S*)-**3**. To our knowledge, (2*S*)-**2** and (2*S*)-**3** are the first examples of the synthesis of amino acid involving a squaryl group with a carbon−carbon bond. Among them, **2** is an analogous amino acid to glutamic acid which plays an important role as an excitatory neurotransmitter in the mammalian central nervous system.[21] Further studies focusing on their biological activities and their incorporation into biologically active peptides are in progress in our laboratory.

Acknowledgment. This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports, and Culture, Japan.

Supporting Information Available: Experimental procedures, spectral data for all compounds, and titration graphs for determination of the pK_a values of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Preliminary pharmacological assays of the synthetic compounds **2** and **3**, for both ionotropic glutamate receptors (KA, AMPA, and NMDA subtypes) in rat brain synaptic membranes and metabotropic glutamate receptors (cloned rat mGluR1, -2, and -4 expressed on CHO cell) were performed. Radioligand binding assays using [^{3}H]KA for KA receptors, [^{3}H]AMPA for AMPA receptors, and [^{3}H]CGP39653 for NMDA receptors revealed that **3** was a potent agonist of KA and AMPA receptors while the magnitude of its activity was slightly weaker than those of glutamate. **3**: KA (IC_{50} = 2.0 μM), AMPA (0.8 μM), and NMDA (31.6 μM). L-Glutamic acid: KA (0.12 μM), AMPA (0.12 μM), and NMDA (0.32 μM). On the other hand, **2** and **3** did not activate mGluRs even at 1 mM concentrations. In addition, they did not inhibit uptake of [^{14}C]glutamate in COS-1 cells expressing human excitatory amino acid transporters (EAAT1 and -2).