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4-Amino-1,2-dithiolane-4-carboxylic Acid (Adt) as Cysteine Conformationally Restricted Analogue. Synthetic Protocol for Adt Containing Peptides

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Abstract—An efficient and versatile protocol to incorporate the achiral and $C^{\alpha,\alpha}$-tetrasubstituted 4-amino-1,2-dithiolane-4-carboxylic acid Adt (**1**) residue into peptides is described. The 2,2-bis[(benzylthio)methyl]glycine *N*-carboxy anhydride (**5**) was found to be the key reactive intermediate from which both Boc-Adt-OMe (**8**) and the glutathione analogue H-Glu(-Adt-Gly-OH)-OH (**12**) can be obtained.

Introduction

Chiral and achiral aminoacids possessing a quaternary α-carbon atom ($C^{\alpha,\alpha}$-tetrasubstituted aminoacids) are the subject of considerable interest. Their incorporation into peptides represents in fact an effective and versatile strategy to restrict the conformational freedom, to stabilize defined secondary structures and to enhance the stability towards chemical and enzymatic hydrolysis.[1] Among these amino acids particular interest has recently been devoted to congeners in which the two C^{α} substituents make part of a ring containing one or two heteroatoms.[2] These heterocyclic $C^{\alpha,\alpha}$-tetrasubstituted models, when inserted into a peptide backbone, combine the tendency to generate specific constraints with the capacity to establish *intra*- or *inter*-molecular non covalent interactions through the side chain heteroatoms. As a consequence of this structural feature some members of this group are actually conformationally restricted mimetics of side chain functionalized proteinogenic amino acids which play crucial roles in binding sites of receptors and enzymes (Fig. 1).

As a prosecution of our interest in the synthesis of glutathione analogues,[3] we were attracted by 4-amino-1,2-dithiolane-4-carboxylic acid (Adt, **1**) as an interesting heterocyclic $C^{\alpha,\alpha}$-tetrasubstituted residue for replacing cysteine in the glutathione as well as in other bioactive peptides. This achiral α-amino acid contains in fact the 1,2-dithiolane ring, a five-membered system which, with varying substituent patterns, is found in a variety of natural and synthetic compounds (Fig. 1).[4,5] 1,2-Dithiolanes can be easily obtained by the corresponding 1,3-dithiols by oxidation with a number of reagents under mild conditions. However, due to an interplay of conformational and electronic effects, connected with the size of the ring, the chemical reactivity of 1,2-dithiolanes is well distinct from that of linear and related cyclic disulfides,[6–8] and represents the key structural feature on which the activity of relevant biomolecules, such as lipoic acid, is based.[4,5,9] Thus, the incorporation of this structural fragment into peptide backbones and the study of the chemical and biochemical consequences of this modification should represent a new and interesting field to be explored.

A literature examination revealed that data referring to Adt are very scarce and actually limited to the synthesis, finalized to the determination of the activity as anti-inflammatory or antiviral agent,[10] and enzymatic inhibitor.[11] In this paper we report a general and efficient protocol to prepare Adt derivatives suitable to incorporate this residue into peptides and their expeditious utilization for the synthesis of a glutathione analogue containing the Adt residue in place of the native *R*-cysteine.

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Chemistry

The strategy followed to obtain Adt derivatives is reported in Scheme 1.[12] The 5,5-bis[(benzylthio)methyl] hydantoin **2** was prepared starting from 1,3-dibromoacetone.[10] The aminoacid **4** was obtained by refluxing **2** with aqueous $Ba(OH)_2$ for eight days.[13] In order to find more rapid and less drastic hydrolysis conditions, the procedure recently reported by Rebek,[14] was explored. This is based on the activation of the hydantoin, towards the nucleophilic ring opening, by *N*-carbamoylation with *tert*-butyloxycarbonyl (Boc) groups. Thus, the bis-Boc derivative **3** was prepared and subjected to milder hydrolytic conditions.[14] In the present case, however, the method failed to give satisfactory results leading in any case to mixtures and partial hydrolysis.[15]

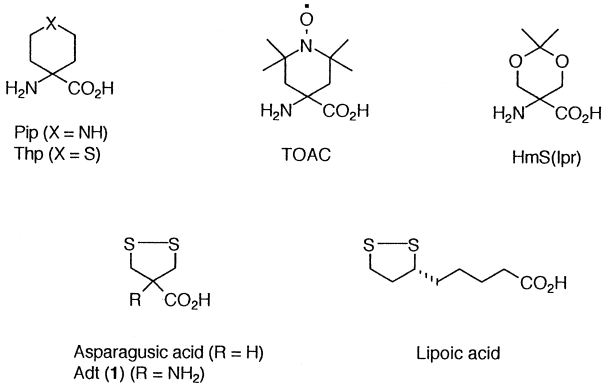
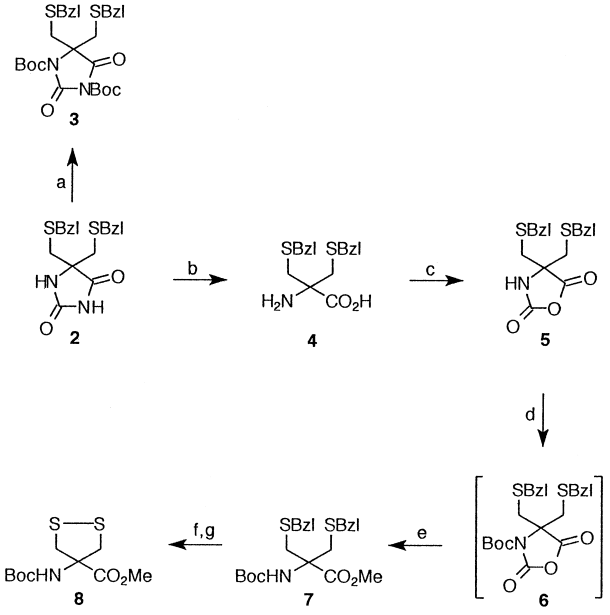


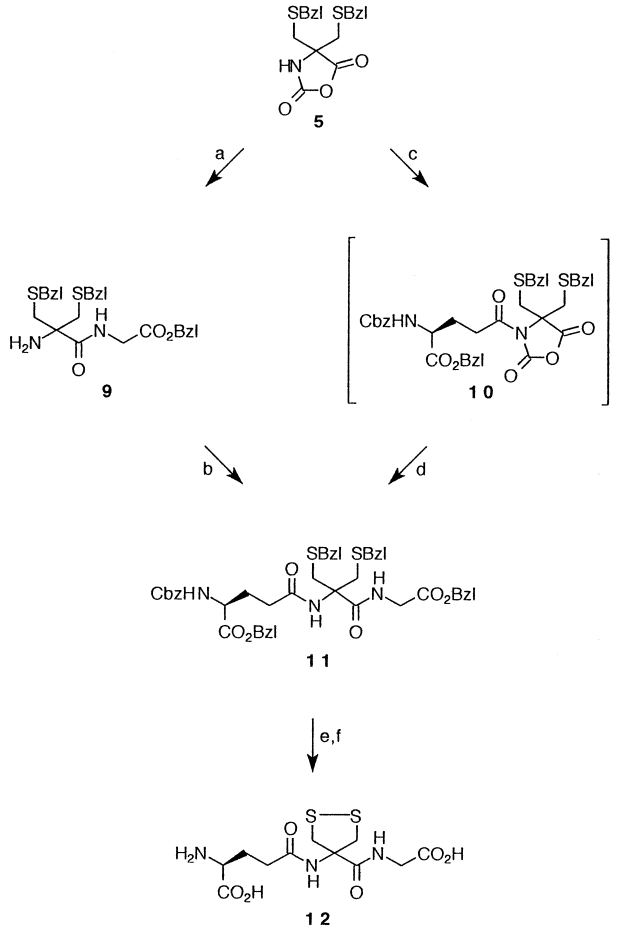
Figure 1. Some representative $C^{\alpha,\alpha}$-tetrasubstituted heterocyclic aminoacids and 1,2-dithiolane derivatives. Abbreviations used: Pip: 4-aminopiperidin-4-carboxylic acid (Ref. 2b); Thp: 4-aminotetrahydrothiopyran-4-carboxylic acid (Ref. 2a); TOAC: 2,2,6,6-tetramethylpyperidin-1-oxyl-4-amino-4-carboxylic acid (Ref. 2e); HmS(Ipr): *O,O*-isopropylidene-α-hydroxymethylserine (Ref. 2c).



Scheme 1. (a) $(Boc)_2O$, DMAP, THF, rt, 2h; (b) Ref. 13; (c) $COCl_2$-toluene, THF, rt, 1 h, 86%; (d) $(Boc)_2O$, Py, THF, rt, 22h; (e) MeOH, NMM, rt, 24 h, 86% (overall from **5**); (f) Na–NH_3 liq., −78 °C; (g) I_2, EtOH, 80% (overall from **7**).

As could be expected, steric hindrance at the C^{α}-carbon atom of the aminoacid **4** renders slow and low yielding the usual amino-protection and carboxy-activation methods. However, a very efficient and flexible procedure was recognized when the sterically hindered aminoacid **4** was converted to the corresponding *N*-carboxyanhydride (NCA, **5**).[16] This intermediate is a stable and crystalline compound which can be cleanly and quantitatively transformed into the *N*-Boc derivative. To achieve this result the NCA **5** was treated with $(Boc)_2O$/pyridine,[17] to give the corresponding urethane protected *N*-carboxyanhydride (UNCA, **6**); this compound is highly reactive and can be quantitatively converted to the methyl ester **7** by treatment at room temperature with methanol in the presence of *N*-methylmorpholine. Deprotection of the two mercapto groups with sodium in liquid ammonia followed by oxidation with iodine gave the orthogonally protected Adt derivative **8** in 69% yield from **5**.

The reactivity evidenced for NCA **5** was then exploited to synthesize an Adt containing glutathione analogue. In Scheme 2[18] two different strategies are reported. In the first route the NCA **5** is used as carboxy activated intermediate of the amino acid **4** and allowed to react



Scheme 2. (a) H-Gly-OBzl·TsOH, TEA, NMM, THF, rt, 24 h, 95%; (b) Cbz-Glu-OBzl, DCC, Py, THF, rt, 90 h, 60%; (c) Cbz-Glu-OBzl, DCC, Py, THF, rt, 24 h; (d) H-Gly-OBzl·TsOH, TEA, NMM, THF, rt, 3 h, 40% (overall from **5**); (e) Na–NH_3 liq., −78 °C; (f) I_2, EtOH, 90% (overall from **11**).

with equimolar quantity of glycine benzyl ester (H-Gly-OBzl) to give the dipeptide benzylester **9** in almost quantitative yield. Contrary to our expectation, the dicyclohexylcarbodiimide (DCC) mediated acylation of the sterically hindered amino group of **9** with *N*-benzyloxycarbonyl-(*S*)-glutamic acid 1-benzyl ester (Cbz-Glu-OBzl) was found to be an efficient step and afforded, although slowly (see Scheme 2), the fully protected glutathione analogue **11** in 60% yield.

It was also possible to obtain the above reported analogue **11** by adopting a different route based on the procedure devised by Shin during the synthesis of oligopeptides containing didehydroaminoacid residues.[19] We found in fact that, in analogy with NCAs of unsaturated amino acids, NCA **5** is efficiently *N*-acylated by *N*-protected amino acids by using DCC in the presence of pyridine; the so-obtained *N*-acyl intermediates can be used in situ for the subsequent nucleophilic ring opening by carboxy-protected aminoacid residues. This procedure was adopted for the synthesis of **11** and is reported in Scheme 2. Here the NCA **5** is acylated with Cbz-Glu-OBzl and the obtained γ-glutamyl-NCA **10** is consecutively used to acylate the amino group of H-Gly-OBzl to afford the fully protected tripeptide derivative **11**. Deprotection and oxidation under the conditions adopted to obtain **8**, afforded the Adt containing glutathione analogue **12**.

Properties of Adt containing peptides together with extension of the studies to other 1,2-dithiolane ring systems, will be reported in due course.

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12. All new compounds were fully characterized. Selected data: **3**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (s, 9H, *t*-butyl), 1.61 (s, 9H, *t*-butyl), 3.06 (ABq, 4H, $J=14.0$ Hz, $2\times CCH_2S$), 3.67 (ABq, 4H, $J=13.2$ Hz, $2\times CH_2C_6H_5$), 7.23–7.35 (m, 10H, aromatics); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 27.8, 28.0 [$2\times C(CH_3)_3$], 35.5, 37.1, ($4\times CH_2S$), 70.5 [$C(CH_2)_2$], 85.1, 86.7 [$2\times C(CH_3)_3$], 127.3, 128.6, 129.0, 137.3 (aromatics), 145.0, 147.9, 148.6, 169.3 ($4\times CO$). **5**: mp 94–95 °C; IR (KBr) 3251, 1839, 1787, 1490, 1451 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.75 (ABq, 4H, $J=14.4$, $2\times CCH_2S$), 3.70 (s, 4H, $2\times CH_2C_6H_5$), 5.49 (br s, 1H, NH), 7.25–7.38 (m, 10H, aromatics); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 36.7 and 37.6 ($4\times CH_2S$), 68.9 [$C(CH_2)_2$], 127.7, 128.9, 129.0, 137.0 (aromatics), 150.8, 170.3 ($2\times CO$). **7**: IR ($CHCl_3$) 3415, 1741, 1708, 1492 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H, *t*-butyl), 2.83 and 3.50 (dd, 4H, $J=13.5$ and $J=13.6$ Hz, $2\times CCH_2S$), 3.67 (ABq, 4H, $J=13.1$ Hz, $2\times CH_2C_6H_5$), 3.70 (s, 3H, OCH_3), 5.91 (br s, 1H, NH), 7.25–7.33 (m, 10H, aromatics); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 28.4 [$C(CH_3)_3$], 36.5, 37.3 ($4\times CH_2S$), 53.0 (OCH_3), 65.5 [$C(CH_2)_2$], 80.0 [$C(CH_3)_3$], 127.1, 128.5, 128.9, 138.2 (aromatics), 154.1, 171.6 ($2\times CO$) **8**: IR (KBr) 3354, 1736, 1707, 1485; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9H, *t*-butyl), 3.37 and 3.63 (dd, 4H, $J=13.0$ and $J=12.9$ Hz, $2\times CH_2$), 3.82 (s, 3H, OCH_3), 5.2 (br s, 1H, NH); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 28.4 [$C(CH_3)_3$], 47.8 ($2\times CH_2S$), 53.5 (OCH_3), 71.6 [$C(CH_2)_2$], 81.1 [$C(CH_3)_3$], 155.1, 171.5 ($2\times CO$); FAB–MS *m*/*e* 279 (M^+).

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18. All compounds were fully characterized. Selected data: **11**: $[\alpha]_D = -7.0°$ (c 1.0, $CHCl_3$); IR ($CHCl_3$) 3375, 1735, 1712, 1673, 1494, 1452 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.83 (m, 1H, Glu β-CH_A), 2.09 (m, 2H, Glu γ-CH_2), 2.25 (m, 1H, Glu β-CH_B), 3.12 (ABq, 2H, $J = 13.8$, CCH_2S), 3.23 (ABq, 2H, $J = 13.7$, CCH_2S), 3.64 (ABq, 4H, $J = 13.3$ Hz, $2\times CH_2C_6H_5$), 3.95 (AB part of ABX system, 2H, $J_{gem} = 19.0$, $J_{vic} = 5.0$ Hz, Gly CH_2), 4.48 (m, 1H, Glu α-CH), 5.12 (m, 6H, $3\times OCH_2$), 5.73 (d, 1H, $J = 8.3$ Hz, Glu NH), 6.12 (1H, s, NH), 7.22–7.34 (m, 25H, aromatics), 7.45 (X part of ABX system, 1H, Gly NH); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 27.9 (Glu β-CH_2), 32.2 (Glu γ-CH_2), 37.0 and 37.8 ($4\times CH_2S$), 41.7 (Gly CH_2), 53.3 (Glu α-CH), 63.0 [$C(CH_2)_2$], 67.1, 67.2, 67.4 (3 x, OCH_2), 127.3, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 129.0, 135.2, 136.1, 138.3 (aromatics), 156.4, 169.4, 171.3, 171.9, 172.0 ($5\times CO$). **12**: $[\alpha]_D = +92.1°$ (c 0.5, H_2O/MeOH 2:1 vol.); IR (KBr) 3371, 1746, 1659, 1547, 1372 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.92 (m, 2H, Glu βCH_2), 2.31 (m, 2H, Glu γ-CH_2), 3.32 (d, 2H, $J = 12.0$ Hz, Adt CH_2), 3.47–3.56 (m, 5H, Adt CH_2, Glu α-CH, and Gly CH_2); ^{13}C NMR (75.43 MHz, D_2O) δ 26.2 (Glu β-CH_2), 31.3 (Glu γ-CH_2), 43.9 (Gly CH_2), 47.8, 47.9 ($2\times CH_2S$), 54.2 (Glu α-CH), 71.8 [$C(CH_2)_2$], 171.9, 174.8, 176.4. ($4\times CO$); FAB–MS *m*/*e* 374 ($M + Na^+$)

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