Pergamon

Tetrahedron Letters 43 (2002) 4861–4864

TETRAHEDRON LETTERS

Unexpected reactions of dithiodiglycolic acid: isolation of a novel seven-membered ring system based on a 2-amino derivative of 7-oxo-6,7-dihydro-[1,4,5]oxadithiepine

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Received 29 March 2002; revised 16 April 2002; accepted 2 May 2002

Abstract—A novel seven-membered ring was unexpectedly isolated from the coupling of dithiodiglycolic acid with 2.2 equiv. of *N*-hydroxysuccinimide, followed by addition of 0.25 equiv. of methyl (4-aminomethyl)benzoate hydrochloride (**2**) in the presence of 2 equiv. of triethylamine. © 2002 Published by Elsevier Science Ltd.

Heterobifunctional reagents can cross-link proteins and other molecules in a stepwise fashion that offers control in the resulting conjugates in terms of their sizes and molar ratios of each component.[1] In our search for a cleavable heterobifunctional cross-linker for protein coupling, we needed to synthesize an intermediate, **4** (Scheme 1). Towards this end, dithiodiglycolic acid was first converted into the corresponding di-*N*-hydroxysuccinimidyl ester (**5**) by treatment with 2.2 equiv. of *N*-hydroxysuccinimide in the presence of 2.2 equiv. of dicyclohexylcarbodiimide (DCC) at room temperature overnight. When the diester formed[2] (**5**) was allowed to react with 0.25 equiv. of methyl 4-(aminomethyl)benzoate hydrochloride (**2**) in DMF containing 2 equiv. of triethylamine (TEA), the desired monoester (**4**) was not obtained. Instead, an unknown compound (**1**) was isolated in 27% yield (based on **2**) among unexpected by-products (Scheme 1, Route a).[3] The structure of the isolated compound, based on spectroscopic analyses (NMR, MS, and IR),[4] was determined to be a derivative of a novel seven-membered ring system, methyl 4-[(7-oxo-6,7-dihydro-[1,4,5]oxadithiepin-2-ylamino)methyl]benzoate (**1**). ^{1}H NMR analysis showed the presence of one equivalent of the 4-(aminomethyl)benzoate moiety. In addition, the two geminal protons of the seven-membered ring occurred at 3.93 and 3.59 ppm as two doublets, respectively, with a coupling constant of 15.8 Hz, which was very typical for a rotation-restrained ring system.[5] High resolution EIMS gave a value of 311.0285 for the molecular ion which matches the calculated value of 311.0286 for the formula of $C_{13}H_{13}NO_4S_2$. The EIMS also revealed the presence of fragmentation peaks compatible with the proposed structure (**1**),[4] among which the peak of 192 is the most abundant one resulting from the loss of a fragment, $COCH_2S_2CH$ (Scheme 2). The latter itself is also present in the spectrum at m/z 119 with a 73% relative intensity.

This novel compound[6] (**1**) is reasonably stable: no significant decomposition was observed when it was refluxed overnight in THF and when its chloroform solution was allowed to stand at room temperature for approximately 3 months. Nevertheless, compound **1**, a nucleophilic enamine, reacted with benzyl bromide at room temperature in the presence of solid K_2CO_3 in acetone to produce **6** in moderate yield as illustrated in Scheme 1.[7]

Since 1 equiv. of **2** was incorporated into the final structure (**1**), it was thought that use of 1 equiv. of **2** in the reaction might increase the yield of the product. Therefore, **2** was allowed to react with **5** in a one to one molar ratio in the presence of 2 equiv. of TEA. It was found that the yield of **1** remained virtually unchanged ($\sim$30%, based on **2**), while the corresponding diamide (**3**), which was undetectable previously when 0.25 equiv. of **2** was employed in the reaction, was formed in about 60% yield (Scheme 1, Route d). In another reaction in which 8 equiv. of TEA[8] was used while the molar ratio between **2** and **5** was maintained at 1:1, it was surprising to find that, under this condition, **1** was not present in the final reaction mixture. Instead, compound **7b** was unexpectedly isolated in 29% yield (Scheme 1, Route c).

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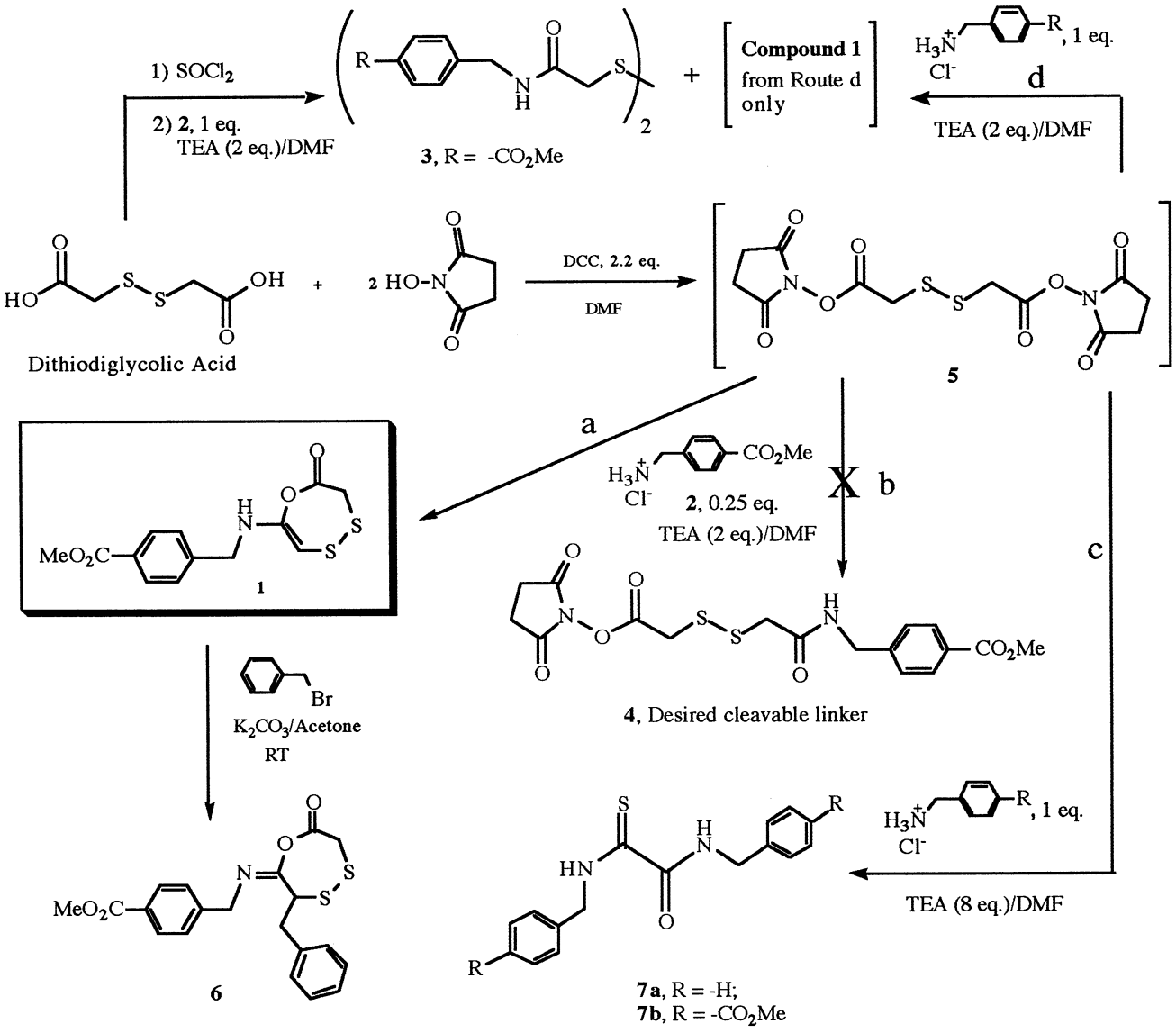
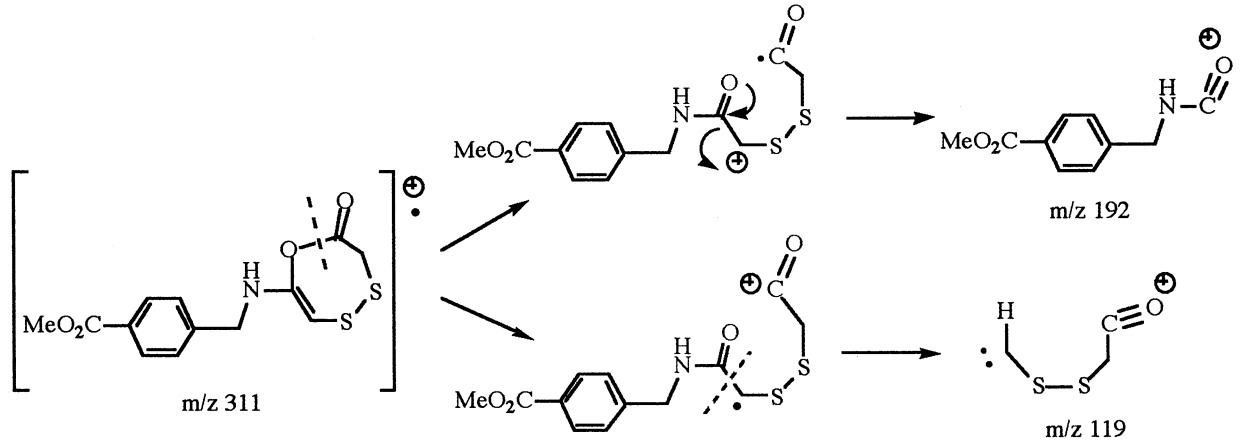
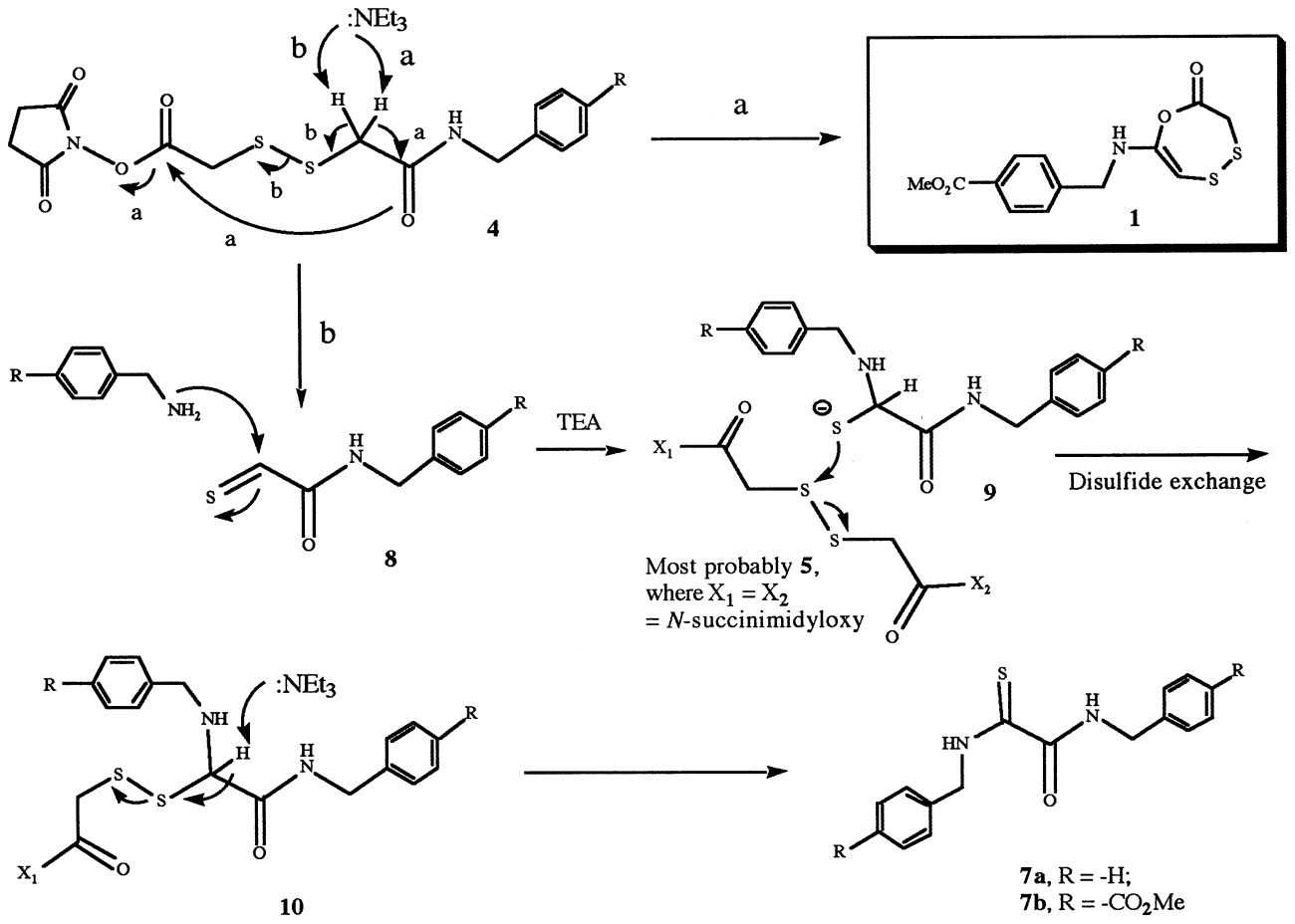
0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)00922-X



Scheme 1.



Scheme 2.



Scheme 3.

When benzylamine hydrochloride was used in the same reaction, the unsubstituted **7a** was obtained in similar yield; structure determination of **7** was based upon NMR, MS, and elemental analysis.[9]

The mechanism for the formation of the unexpected compounds (**1** and **7**) is proposed in Scheme 3. Presumably, the diester (**5**, Scheme 1) was formed initially and it was able to react with **2** to give the desired product (**4**). In the presence of a moderate amount of TEA, however, a ring closure probably occurred rather easily through displacement of *N*-hydroxysuccinimide (Scheme 3, Route a) to yield the compound (**1**) containing a novel seven-membered ring. The role of the *N*-succinimidyloxy ester appears to be critical. In one reaction, when dithiodiglycolic acid was treated with DCC alone and then allowed to react with **2**, **1** was not formed. Yet in another reaction, when dithiodiglycolic dichloride was treated with **2** in the presence of TEA, the only product isolated was the corresponding diamide (**3**, Scheme 1).

When the amount of TEA was increased from 2 equiv. to 8 equiv., the monoester (**4**) may be cleaved by the excess TEA to give the α-ketothioaldehyde (**8**, Scheme 3, Route b), which could then be attacked by a second molecule of 4-(aminomethyl)benzoate to form the thiolate **9** in the presence of excess TEA. The thiolate (**9**) would give rise to **10**, through a disulfide exchange (probably with **5**). TEA abstraction of the proton α to the carbonyl group in **10** should result in the formation of **7**. The unsubstituted compound, **7a**, was known in the literature[10] and was used as a complexing reagent for quantitative analysis of Pt (II) and Pd (II).[11]

In summary, a novel seven-membered ring system (**1**) was unexpectedly isolated. Its reactivity and potential utilities (e.g. for use as a novel scaffold in drug design) deserve further investigation.

References

1. The heterobifunctional reagents including the ones with cleavability have been comprehensively reviewed in these two books: (a) Wong, S. S. *Chemistry of Protein Conjugation and Cross-Linking*; CRC: Boca Raton, FL, 1991; (b) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: San Diego, CA, 1996.
2. ^{1}H NMR spectrum of **5** ($CDCl_3$): δ 3.90 (s, 4H), 2.67 (s, 8H).
3. Overall reaction procedure (protected from moisture throughout the reaction): to a mixture of dithiodiglycolic acid (2.19 g, 12.0 mmol), *N*-hydroxysuccinimide (3.04 g, 26.4 mmol), and DCC (5.45 g, 26.4 mmol) was added 120 mL of DMF. The resulting reaction mixture was stirred at room temperature overnight. A small aliquot was withdrawn from the mixture, filtered, and then evaporated in vacuo. The residue was dissolved in $CDCl_3$ for

^{1}H NMR recording and the result is shown in Ref. 2. To the remaining majority of the reaction mixture was added, dropwise, a mixture of **2** (605 mg, 3.0 mmol) and TEA (3.35 mL, 24 mmol) in 30 mL of DMF. After stirring at room temperature for 5 h, the mixture was filtered and the filtrate evaporated in vacuo. The residue was then loaded onto a silica gel column and eluted with CH_2Cl_2/MeOH (9/1, v/v). The fast-eluting fractions ($R_f \sim 0.8$) were further purified on another silica gel column and the elution was effected with hexane/ethyl acetate (1/1, v/v). The fractions containing the $R_f \sim 0.5$ component were pooled and evaporated in vacuo to give **1**.

4. For the characterization of **1**, the following analyses were performed: ^{1}H NMR spectrum ($CDCl_3$): δ 7.97 (d, 2H, $J=8.4$ Hz), 7.30 (d, 2H, $J=8.4$ Hz), 7.06 (t, 1H, $J=5.8$ Hz), 5.11 (s, 1H), 4.50 (d, 2H, $J=5.8$ Hz), 3.93 (d, 1H, $J=15.8$ Hz), 3.89 (s, 3H), 3.59 (d, 1H, $J=15.8$ Hz). EIMS m/z (ion, relative intensity) 311.0 (M^+, 17), 279.1 (M–$HOCH_3$, 22), 238.1 (M–COCHS, 23), 206.1 (M–$COCHS_2$, 14), 192.1 (M–$COCH_2S_2CH$, 100), 164.1 ($CH_3O_2CC_6H_4CH_2NH$, 22), 149.1 ($CH_3O_2CC_6H_4CH_2$, 96), 119.0 ($COCH_2S_2CH$, 73). EIMS (high resolution) m/z 311.0285; Calcd for $C_{13}H_{13}O_4NS_2$, 311.0286. IR 3288, 1723, 1646, 1282 cm^{-1}.
5. For example, similar situation is observed in the seven-membered ring system of 3-unsubstituted benzodiazepines.
6. A thorough search of CAS database indicated that this seven-membered ring system had never been reported before.
7. ^{1}H NMR spectrum of **6** ($CDCl_3$): δ 7.97 (d, 2H, $J=8.4$ Hz), 7.40–7.25 (m, 7H), 5.01 (s, 2H), 4.38 (d, 1H, $J=2.1$ Hz), 4.17 (d, 1H, $J=17.4$ Hz), 3.88 (s, 3H), 3.80 (s, 2H), 3.42 (dd, 1H, $J_1=17.4$, $J_2=2.1$ Hz).
8. In the original reaction,[3] the molar ratio of TEA vs. aminomethylbenzoate (**2**) is also 8 to 1.
9. **7a**: ^{1}H NMR ($CDCl_3$): δ 9.70 (s, 1H, broad), 8.55 (s, 1H, broad), 7.40–7.25 (m, 10H), 4.82 (d, 2H, $J=5.8$ Hz), 4.52 (d, 2H, $J=6.2$ Hz). ^{13}C NMR ($CDCl_3$) δ 186.08, 158.40, 136.79, 135.01, 128.99, 128.83, 128.35, 127.82, 127.69, 50.19, 44.70. EIMS (high resolution) m/z 284.0988; Calcd for $C_{16}H_{16}N_2OS$, 284.0983. Anal. Calcd for $C_{16}H_{16}N_2OS$: C, 67.58; H, 5.67; N, 9.85; S, 11.27. Found: C, 67.18; H, 5.58; N, 9.66; S, 11.14. **7b**: ^{1}H NMR (DMSO-d_6) δ 11.35 (t, 1H, $J=6.2$ Hz), 9.32 (t, 1H, $J=6.2$ Hz), 7.90 (d, 4H, $J=7.9$ Hz), 7.40 (d, 4H, $J=7.9$ Hz), 4.88 (d, 2H, $J=6.2$ Hz), 4.46 (d, 2H, $J=6.2$ Hz), 3.82 (s, 6H).
10. Duffin, K. *J. Chem. Soc.* **1956**, 3189.
11. Li, Z. *Yejin Fenxi* **1993**, *13*, 25–28. (CA 122:204006).