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EDC-mediated condensations of 1-chloro-5-hydrazino-9,10-anthracenedione, 1-hydrazino-9,10-anthracenedione, and the corresponding anthrapyrazoles

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Abstract—The EDC-mediated condensation of 1-chloro-5-hydrazino-9,10-anthracenedione afforded an N-1 acyl anthrapyrazole instead of the expected hydrazide. The regiochemistry of the *N*-acyl substituent was assigned on the basis of an extensive set of NMR experiments, and identification of this isomer suggests a reaction sequence based on initial acylation and subsequent cyclization. In contrast, the parallel reaction of 1-hydrazino-9,10-anthracenedione proceeded to afford the expected hydrazide.

The neuronal ceroid lipofuscinosis (NCLs), also known as Batten disease, are a group of rare but devastating diseases characterized by progressive neurodegeneration.[1] One form, known as late infantile neuronal ceroid lipofuscinosis (or LINCL), is caused by inactivating mutations in the lysosomal enzyme tripeptidyl protease I (TPP-I).[2,3] Current therapy for LINCL can only relieve the seizure disorder and cannot cure the underlying cause or slow the progression of the disease.[1,2] Because TPP-I is a lysosomal enzyme that can be secreted from cells where it is over expressed and taken up by cells where it is deficient, LINCL may be amenable to development of gene-based therapies. However, investigations of potential gene therapies for this disease are critically dependent upon advances in histochemical methods for detection of TPP-I activity in tissue sections. In this context, we recently reported[4] the preparation and partial characterization of several tripeptides prepared from anthraquinone derivatives. Because these compounds have been shown to be useful as histochemical probes for TPP-I activity,[4] their complete characterization has become important. In this Letter, we report the regiochemistry observed in EDC-mediated coupling reactions with these substrates, and the spectroscopic studies that allow complete structural assignments to the products.

According to Dikov et al.,[5] 1-chloro-5-hydrazino-9,10-anthracenedione (**1**) was converted to the alanine derivative **2** through DCC-mediated coupling with Boc-L-alanine, and the product was converted to the tripeptide derivative **3** through a sequence of coupling and deprotection steps (Scheme 1). In our hands, reaction of 1,5-dichloro-9,10-anthracenedione (**4**) with hydrazine smoothly affords the hydrazine derivative **1** (Scheme 2).[6–10] However, attempted coupling of compound **1** with Boc-L-alanine in a DCC-mediated reaction gave a coupled product only in low yield. The same compound was obtained in much better yield when 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was employed as the coupling agent. The carbodiimide procedure for formation of the amide bond has been well established,[11] and DCC frequently has been used as a coupling reagent. However, the resulting urea and acylurea by-products may have solubility properties similar to the peptides, so the desired products can be hard to isolate.[12] With the water soluble EDC reagent, the by-products are readily removed by extraction with water, which may explain the improved yield.[13]

The EDC-mediated condensation of the substituted hydrazine **1** with Boc-L-alanine gave a single *N*-acylated product as a dark red solid in 65% yield.[7,9] The product initially was assumed to be the amide **2** as previously described.[5] However careful analysis of the NMR spectra did not support that assignment. Of special significance is the ^{13}C NMR data.[14] The symmetrical anthraquinone **4** shows a single resonance at 180.9 ppm

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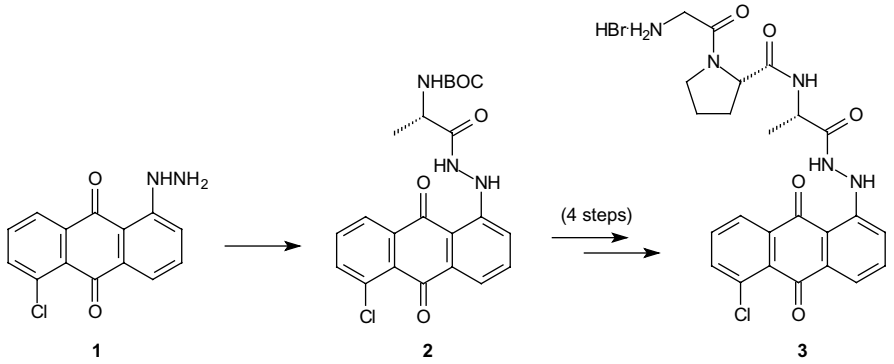
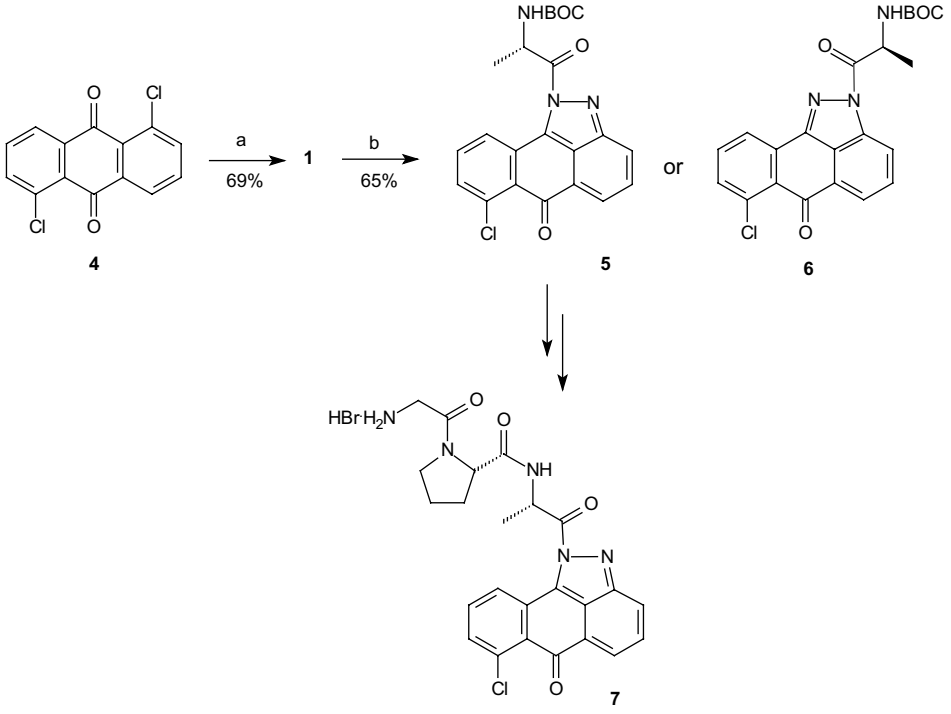


Scheme 1.



Scheme 2. Reagents and conditions: (a) $NH_2NH_2 \cdot H_2O$, Pyr, reflux, 3 h; (b) Boc-L-alanine, EDC, HOBt, NMM, THF.

that can be assigned to the two quinone carbonyl carbons. In the hydrazine derivative **1** this symmetry is lost, and two distinct quinone resonances are observed at 183.8 and 182.5 ppm. In contrast, in the product of the DCC and EDC condensations with Boc-L-alanine, only one quinone-type resonance is apparent (181.1 ppm) and, because the expected total of 16 aromatic or carbonyl resonances is observed, it is not possible that an accidental overlap of two resonances has occurred. Instead it appears probable that cyclization to a pyrazole derivative occurred under these conditions, as previously observed with alkyl-substituted hydrazines derived from related anthraquinones.[9] It was not immediately clear whether this product was the N-1 acyl pyrazole **5** or the isomeric pyrazole **6** and there is precedent for both regioisomers.[15] Formation of compound **5** could be envisioned through a reaction sequence where acylation of the more nucleophilic terminal nitrogen was followed by condensation, but this would require addition of the hydrazide nitrogen to the quinone carbonyl. On the other hand, if cyclization precedes ring closure, formation of either isomer **5** or **6** may be possible.[15]

The condensation product was assigned structure **5** through extensive analysis of its spectral data, and especially the HMBC, HMQC, and NOESY data summarized in Figure 1. A key HMBC correlation between the carbonyl carbon observed at 181.1 ppm and the H-5 resonance at 8.04 ppm allowed assignment of hydrogens and carbons on the C-ring through HMQC and COSY experiments. Two carbons adjacent to the pyrazole nitrogens were assigned by interpretation of a correlation between the C-8 carbon resonance at 137.9 ppm and the C-6 hydrogen observed at 7.77 ppm, and a second correlation between the C-9 carbon at 144.1 ppm and the C-1 hydrogen at 8.24 ppm.

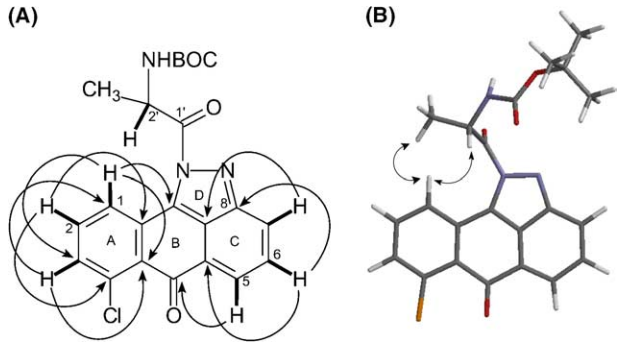
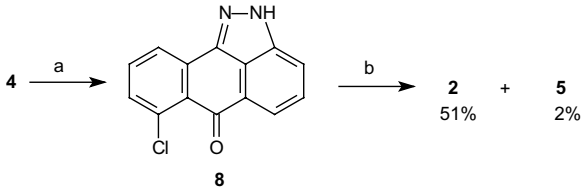


Figure 1. (A) Key HMQC (—) and HMBC (↷) correlations and (B) NOESY correlations for compound **5**.

The final assignment of the product as structure **5** also was supported by a combination of COSY and NOESY experiments. For example, compound **5** showed strong NOESY correlations between both the α hydrogen of the alanine moiety (5.74 ppm) and the alanine methyl group (1.65 ppm) and the C-1 anthraquinone hydrogen (8.24 ppm, Fig. 1). This strong correlation provides clear support for assignment of the structure as presented in Figure 1, especially when considered with the energy minimized structure as shown (PM3 level).

Because Dikov's report[5] contained no spectral data and only a very brief summary of the reactions employed, it is not possible to establish whether their reported biological evaluations were based on a tripeptide derived from a quinone or a pyrazole. In our study,[4] the pyrazole derivative **5** was carried on to the corresponding tripeptide **7** through standard methods (Scheme 2), and shown to be a useful probe for TPP-I activity.[4]

Several experiments were conducted to illuminate the reaction sequence that leads to compound **5**. Extended heating of compound **4** with hydrazine in the presence of *i*Pr_2NEt leads to the pyrazole **8** (Scheme 3).[7] When compound **8** was treated with Boc-L-alanine under standard coupling conditions followed by an aqueous work-up, the major product could be assigned as the amide **2** given that two quinone carbonyl resonances were observed in the ^{13}C NMR spectrum (183.2 and 181.4 ppm). The minor product of this reaction proved to be identical to the material prepared earlier (i.e., pyrazole **5**). Compound **1** also was treated with a carbodiimide (DCC or EDC), *N*-methylmorpholine (NMM), and 1-hydroxylbenzotriazole (HOBt) in THF in the absence of Boc-L-alanine. These reactions afforded only recovered starting material; none of the pyrazole **8** was detected under these conditions. Taken together, these experiments suggest that compound **5** is formed from the substituted hydrazine **1** through a reaction sequence where N-acylation of the terminal nitrogen is followed by formation of the pyrazole ring system.



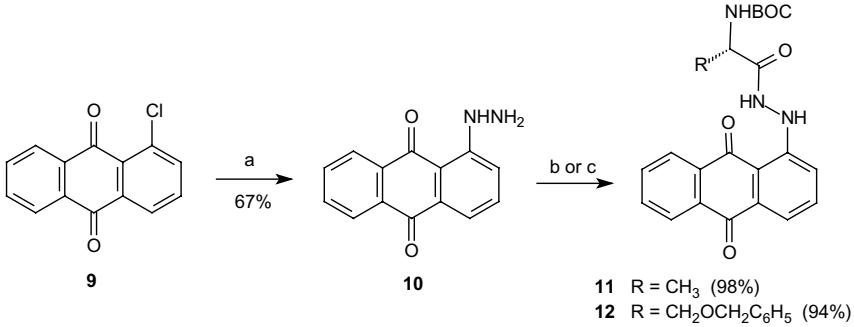
Scheme 3. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, *i*Pr_2NEt, reflux; (b) Boc-L-alanine, EDC, HOBt, NMM, THF.

Parallel studies with the related anthraquinone **9** have shown that relatively brief treatment with hydrazine affords the aryl hydrazine derivative **10** (Scheme 4), and longer reaction time gives the anthrapyrazole **13** (Scheme 5). Treatment of compound **10** with EDC and Boc-L-alanine clearly gave compound **11** rather than a pyrazole product, as evidenced by observation of two quinone carbon resonances at 185.3 and 182.9 ppm.[16] The parallel reaction with a protected serine provides the analogous product **12**.

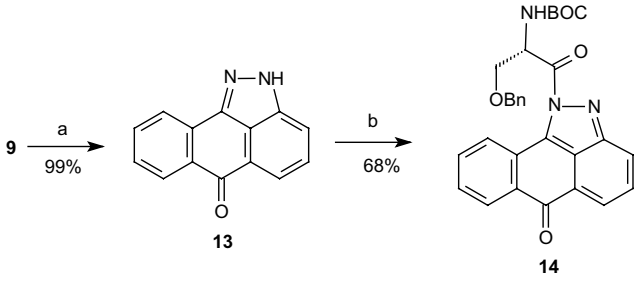
N-Acylation of compound **13** under the reaction conditions employed with compound **8** afforded the pyrazole derivative **14** in 68% yield (Scheme 5). Assignment of a pyrazole product was relatively straightforward based on observation of a single quinone carbonyl resonance at 182.2 ppm. The regiochemistry of this reaction was established through extensive analysis of the NMR spectra. As shown in Figure 2, key HMBC correlations were observed between the C-4 hydrogen at 8.32 ppm, as well as the C-5 hydrogen at 8.03 ppm, and the carbonyl carbon at 182.2 ppm. These correlations allowed assignment of hydrogens and carbons in the A- and C-rings through HMQC and COSY data. The A-ring has four aromatic hydrogens and the C-ring has just three, so COSY and HMQC correlations readily allowed the assignment of all hydrogens and carbons in these two ring systems. The two aromatic carbons adjacent to the pyrazole nitrogens were assigned by interpretation of a correlation between the C-8 carbon at 138.0 ppm and the C-6 hydrogen at 7.72 ppm and a second correlation between the C-9 carbon at 144.4 ppm and the C-1 hydrogen at 8.08 ppm.

The final structure assignment of compound **14** was based on a combination of COSY and NOESY experiments. Compound **14** showed strong NOESY correlations between the α hydrogen at 5.78 ppm and one of the –CH_2O– hydrogens (at 4.20 ppm) and the C-1 hydrogen of the aromatic ring (Fig. 2). These correlations clearly support assignment as the N-1 derivative shown rather than the N-2 isomer.

In summary, the EDC-mediated coupling of the chloroanthraquinone derivative **1** with Boc-L-alanine proceeds to afford the N-1 acyl pyrazole **5**, while the parallel EDC-mediated condensation of pyrazole **8** followed by work-up with aqueous acid gives the ring-opened product **2**. In the absence of the chloro substituent, different reactivity is observed. The EDC-mediated condensation of hydrazine **10** proceeds without pyrazole formation and gives the hydrazide **11**, and condensation of the pyrazole **13** gives the N-1 acyl pyrazole **14**. While determination of the ways that the presence or absence of a remote chloro substituent influences these reactions will require further investigation, this work establishes



Scheme 4. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, Pyr, reflux, 3 h; (b) Boc-L-alanine, EDC, HOBt, NMM, THF; (c) Boc-*O*-benzyl-L-serine, EDC, HOBt, NMM, THF.



Scheme 5. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, Pyr, reflux, 9 h; (b) Boc-*O*-benzyl-L-serine, EDC, HOBt, NMM, THF.

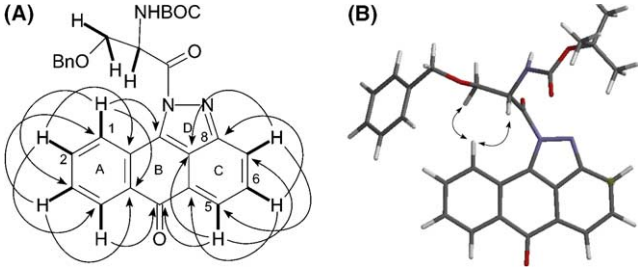


Figure 2. (A) Key HMQC (▬) and HMBC (↷) correlations and (B) NOESY correlations for compound **14**.

the structures of the condensation products and will advance their use as probes of TPP-1 distribution following gene-transfer experiments.

Acknowledgements

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14. For compound **5**: ^{1}H NMR ($CDCl_3$) δ 8.39 (d, $J = 8.1$ Hz, 1H), 8.24 (dd, $J = 6.6$, 2.2 Hz, 1H), 8.04 (d, $J = 7.3$ Hz, 1H), 7.77 (t, $J = 7.8$ Hz, 1H), 7.64–7.57 (m, 2H), 5.76–5.66 (m, 1H), 5.44 (d, $J = 8.1$ Hz, 1H), 1.65 (d, $J = 6.9$ Hz, 3H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 181.1, 173.6, 155.1, 144.1, 137.9, 137.3, 135.0, 133.2, 132.2, 131.6, 129.6, 126.4, 124.6, 123.6, 123.1, 120.7, 80.1, 49.2, 28.2 (3C), 19.2; HRMS (FAB) calcd for $C_{22}H_{21}N_3O_4Cl$ $(M+H)^+$ 426.1221, found 426.1226.
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16. For compound **11**: ^{1}H NMR ($CDCl_3$) δ 10.54 (s, 1H, exchanges with D_2O), 8.31–8.21 (m, 3H), 7.78–7.71 (m, 3H), 7.42–7.30 (m, 5H), 7.23 (d, $J = 8.5$ Hz, 1H), 5.46 (d, $J = 6.7$ Hz, 1H), 4.64 (d, $J = 11.7$ Hz, 1H), 4.59 (d, $J = 11.7$ Hz, 1H), 4.51–4.48 (m, 1H), 4.00 (dd, $J = 8.8$, 3.6 Hz, 1H), 3.70 (dd, $J = 9.0$, 6.4 Hz, 1H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 185.3, 182.9, 170.4, 155.5, 151.0, 137.1, 135.1, 134.1, 134.0, 133.8, 133.3, 132.6, 128.5 (2C), 128.0, 127.9 (2C), 126.8, 126.6, 118.4, 118.0, 114.4, 80.7, 73.7, 69.7, 53.4, 28.3 (3C); HRMS (FAB) calcd for $C_{29}H_{30}N_3O_6$ $(M+H)^+$ 516.2135, found 516.2129.