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Solid-phase synthesis of 2-amino-3-chloro-5- and 8-nitro-1,4-naphthoquinones: a new and general colorimetric test for resin-bound amines

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Abstract—Treatment of resin-bound primary or secondary alkyl and arylamines with 2,3-dichloro-5-nitro-1,4-naphthoquinone leads to the rapid formation of intensely colored (red) beads. The resulting 2-amino-3-chloro-5- and 8-nitro-1,4-naphthoquinones can be cleaved rapidly from acid-labile supports in high yields and purities. The reaction is of value as a sensitive and general qualitative test for amino groups on-resin that can be followed by cleavage for characterization and quantification of the chromogen(s) responsible for the color.

Over the past decade solid phase synthesis[1] has been applied widely but difficulties in monitoring the progress of reactions remain a barrier toward more widespread use. While colorimetric tests for resin-bound functional groups[2–6] are of value, those for amino groups have their limitations. The Kaiser ninhydrin test[7] is limited to primary aliphatic amines and the harsh reaction conditions can lead to false positives. Ion pairing with a colored anion[8] is sensitive but this test will give positive results for any resin-bound base. Likewise, NPIT[9] reacts with resin-bound alkyl- and arylamines; however, amine quantification relies on the subsequent release of the dimethoxytrityl cation and thus two or more amines on the resin cannot be differentiated. Other tests use colored precursors[10] making the color of the resin difficult to distinguish from that of the solution. The colorimetric test for secondary amines involving reaction with chloranil and acetaldehyde[11,12] is assumed, on the basis of solution phase precedent,[13,14] to give enamine quinones. Recently, an additional test,[15] in which resin-bound arylamines form red products on treatment with chloranil (in the absence of acetaldehyde) at 100 °C, has been reported. Both these tests are qualitative since the chromogens responsible for the color have not been cleaved for characterization or quantification. This paper describes the reaction of weakly colored 2,3-dihalo-1,4-naphthoquinones with resin-bound amines to give highly colored beads that release the chromogenic 2-amino-3-chloro-1,4-naphthoquinone derivatives on treatment with TFA.

Reaction of 2,3-dihalo-1,4-naphthoquinones, **1**–bf 3 with primary amines results in the displacement of one chlorine to give 2-amino-3-chloro-1,4-naphthoquinones.[16] While quinones **1**–**3** are pale yellow in solution due to tailing of a n–π* absorption into the visible region, the introduction of the amino group creates π–π* donor–acceptor chromogens[17] which absorb more intensely in the 450–500 nM region of the visible spectrum and are thus orange to red in color.[16]

Treatment of Rink amide (RAM)[18] resin with saturated (ca. 200 mM) solutions of **1** or **2** in either DMF or CH_2Cl_2 in the presence of 2,6-di-*tert*-butylpyridine as base, afforded deep red resins **4**-RAM and **5**-RAM (Scheme 1). These resin-bound quinones are, in essence, vinylogous amides, and it was thus of interest to subject them to typical amide cleavage conditions. In fact, amino quinones **7**[19] and **8**[20] were isolated in high purities after treatment with TFA/CH_2Cl_2 and good yields of analytically pure products were obtained after filtration through silica gel (Table 1, entries 1 and 2). In order to

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Keywords: 1,4-Naphthoquinone; Solid-phase synthesis; Colorimetric test.

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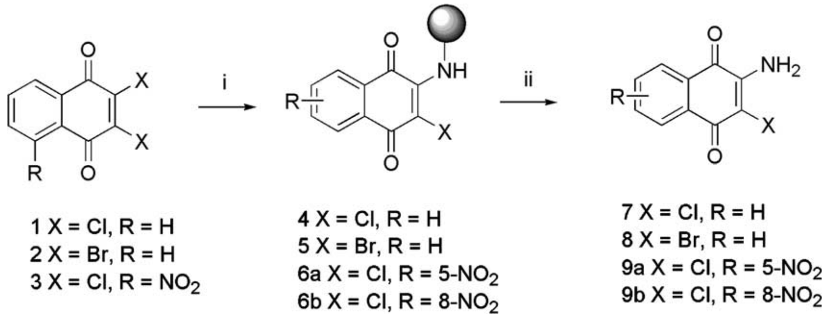


Scheme 1. Reagents and conditions: (i) Resin–NH_2, 2,6-di-*tert*-butylpyridine, 25 °C, 2 h; (ii) TFA–CH_2Cl_2 (1:1), 25 °C.

Table 1.

Entry	Resin	Product	Purity[a] (%)	Yield[b] (%)
1	RAM	**7**	85	75
2	RAM	**8**	95	70
3	RAM	**9**	95	90
4	RAM plug	**9**	95	95
5	PAL	**9**	75	75
6	MBHA	**9**	95	55
7	**11**R = Me	**13**	90	50
8	**11**R = *n*-Pr	**14**	95	70
9	**11**R = phenethyl	**15**	94	50
10	**11**R = *i*-Pr	**16**	90	35
11	**11**R = Ph	**17**	50	30
12	Wang-NH-*n*-Pr	**14**	95	40

[a] LC–MS analysis (see Supplementary data).

[b] Isolated yield after chromatography.

develop an improved substrate, further reactions were conducted using the 5-nitro compound **3** which is more reactive toward amines in solution phase reactions affording regioisomeric mixtures of mono-substitution products that have higher extinction coefficients than the parent compounds[16] and is approximately 2-fold more soluble than **1** and **2** in DMF and CH_2Cl_2. After removal of the Fmoc group from commercially available RAM resin by treatment with piperidine–DMF (1:4),[21] reaction with **3** led to the instantaneous formation of deep red beads of **6**-RAM clearly visible in the yellow solution of **3**. Cleavage was also rapid, two 15 min treatments with TFA–CH_2Cl_2 (1:1) affording the regioisomeric mixture of amino-quinones **9** in high yield and purity (entry 3). Similar results were obtained with RAM resin in the plug^{TM} format.[22] The amine functionalized resins PAL[23] and MBHA[24] (Fig. 1) were also allowed to react with quinone **3**. On a preparative scale, reactions were allowed to proceed for 2 h at ambient temperatures although intensely colored resins were observed within seconds.[25] Subsequent treatment with TFA/CH_2Cl_2 (1:1) afforded **9** in good yields and purities. These cleavage conditions are noteworthy in the case of MBHA resin for which the standard conditions for release of amides are usually TFMSA or HF.[24]

The reaction of quinone **3** with resin-bound secondary amines was next investigated. Several primary amines of variable steric and electronic properties were anchored to FDMP resin (Fig. 1)[26] under reducing conditions affording resins **11** (Scheme 2). The resultant alkyl derivatives were found to react rapidly with quinone **3** in the presence of 2,6-di-*tert*-butylpyridine to give the red resin-bound quinones **12**. Treatment with TFA:CH_2Cl_2 (1:4) led to very rapid cleavage of quinones **13**–**16** as regioisomeric mixtures in high purities.[27] The isolated yields listed in Table 1 are based on two steps and thus also reflect the relative efficiencies of the reductive amination steps. The regioisomers were formed in approximately equal amounts and were readily separated by chromatography on neutral alumina. In the case of mixture **14**, the higher R_f component was shown to be the 5-nitro isomer **14a** and the lower R_f component the 8-nitro isomer **14b** by ^1H–^{13}C (HMBC) analysis. For comparison, *n*-propylamine was anchored to a resin with a less electron-rich linker by reaction with Wang chloride (Fig. 1). Subsequent reaction with **3**, followed by cleavage also gave **14a/b** in high purity and yield (entry 12). While reactions were allowed to proceed for longer periods on a preparative scale it was possible to conduct a 5 min qualitative test in which alkyl derivatives of resin **11** were allowed to react with **3** for 2 min followed by washing, cleavage for 2 min, and TLC analysis on neutral alumina. Deep red beads that afford a red cleavage solution on treatment with TFA which, on TLC analy-

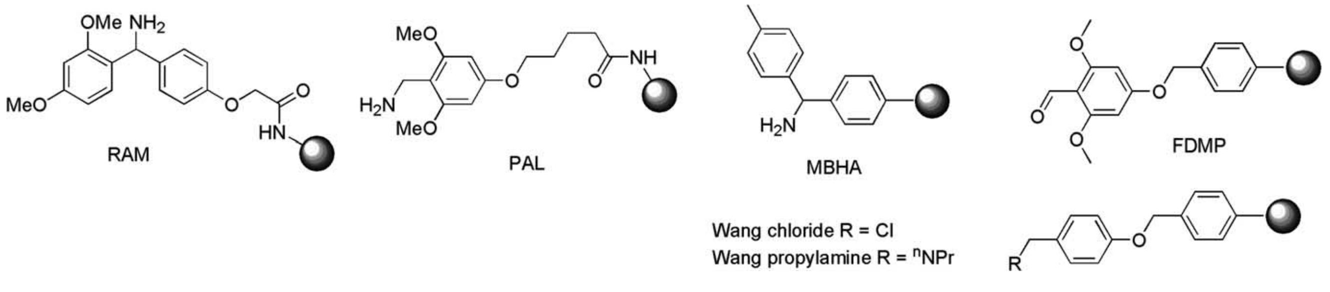
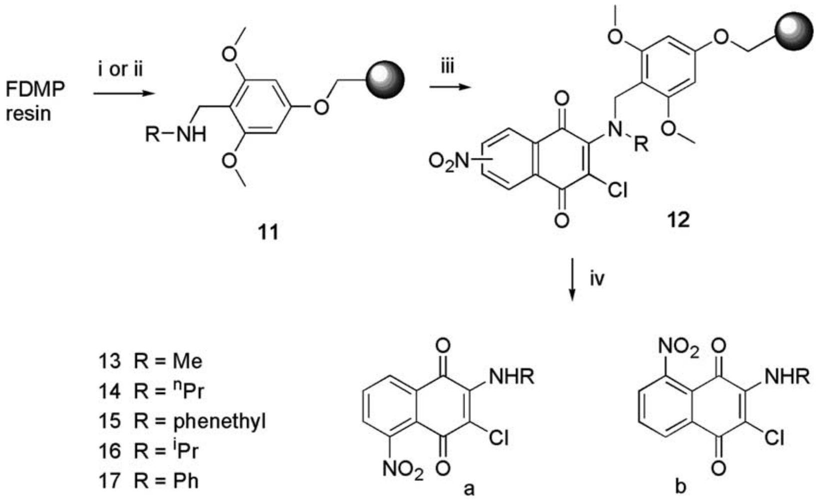


Figure 1. Polystyrene-based resins described in the text.



Scheme 2. Reagents and conditions: (i) RNH_2, HOAc, $NaBH(OAc)_3$, DMF, 25 °C, 16 h; (ii) $ArNH_2$, HOAc, $NaBH_3CN$, DMF, 25 °C, 16 h; (iii) **3**, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, 25 °C, 2 h; (iv) TFA–CH_2Cl_2 (1:1), 25 °C, 0.25 h.

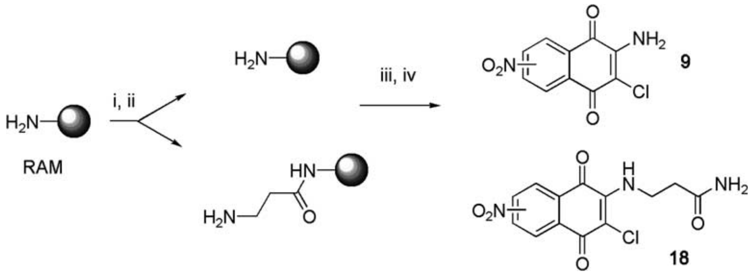
sis, affords two red bands of approximately equal intensities constitutes a positive test for secondary amines. It should be noted that prior to reaction with **3**, the amines are not readily cleaved from resins **11**. In the case of resin **11** (R = Ph),[6] reaction with **3** was noticeably slower. However, heating under microwave irradiation[28] for 10 min in dichloroethane at 80 °C afforded intensely red beads of **12** (R = Ph), cleavage of which afforded **17** in moderate purity.

To assess the value of quinone **3** for monitoring a typical solid-phase synthesis, several coupling reactions between N^{α}-Fmoc-β-Ala-OH and RAM resin were conducted in which the mole ratio of DIC was varied from 0.1 to 4 equiv (Scheme 3). The (partially) coupled resins were deprotected with piperidine then treated with **3** and 2,6-di-*tert*-butylpyridine for 1 h at ambient temperature and cleaved. After cleavage, the coupling involving the use of 4 equiv of DIC afforded **18** in 80% yield.

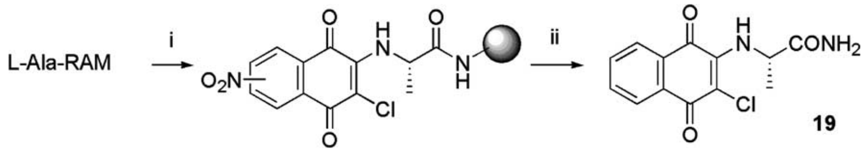
TLC analysis of the remaining cleavage products showed the presence of isomeric pair **9** (two closely eluting orange spots) and the β-Ala derivatives **18** (two closely eluting red bands) the relative amounts of which were in accord with the amount of DIC used in the coupling step. To assess the sensitivity of this colorimetric test, mixtures of $^{\alpha}N$-Fmoc–Ala-OH and 3-(3,4-dimethoxy-phenyl)-propionic acid in various mole ratios were coupled to RAM resin, and following Fmoc deprotection, allowed to react with **3** in the presence of 2,6-di-*tert*-butylpyridine. A coupling reaction in which the respective mole ratios were 1:99 afforded pink beads that were cleaved and analyzed by TLC showing the presence of **18**; thus, the sensitivity of the test is <10 μmol/g resin.[29]

Reaction of **3** with H-Ala-RAM resin proved to be slower than that with the analogous β-Ala-RAM presumably due to both inductive and steric effects. Although orange beads were observed within a few minutes at ambient temperature, overnight reactions were necessary in order to obtain moderate yields of **19** following cleavage. However, microwave irradiation of the reaction mixture for 10 min at 150 °C in dichloroethane led to the formation of intensely red beads from which **19** was isolated following cleavage in 90% yield and 85% purity (Scheme 4).

In conclusion, this paper describes the reaction of 2,3-dichloro-5-nitro-1,4-naphthoquinone (**3**) with resin-bound amines to give highly colored products rapidly with high sensitivity. The resulting 2-amino-1,4-naphthoquinones can be cleaved from acid-labile resins in high yields and purities. The chromogenic products derived from secondary amines are activated toward cleavage and afford intensely colored solutions within seconds that can be analyzed rapidly by TLC, LC–MS or visible spectroscopy. The test provided by Scheme 2 improves on the



Scheme 3. Reagents and conditions: (i) Fmoc-β-Ala-OH, DIEA, DIC, HOBt, DMAP, DMF, 25 °C; (ii) piperidine–DMF (1:4), 0.25 h (iii) **3**, 2,6-di-*t*-butylpyridine, CH_2Cl_2, 25 °C, 2 h; (iv) TFA–CH_2Cl_2, (1:1) 25 °C, 0.25 h.



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Scheme 4. Reagents and conditions: (i) **3**, 2,6-di-*tert*-butylpyridine, $C_2H_2Cl_2$, 150 °C, µWI; (ii) TFA–CH_2Cl_2 (1:1).

chloranil test (the products from which are hydrolytically unstable and cannot be cleaved) and is complementary to the 2,4-DNP[5,6] test for resin-bound aldehyde groups. Further work on the quantitative aspects of the test is in progress and will be reported in due course.

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Supplementary data

Synthetic details for the solid-phase synthesis of compounds **7**, **8**, and **9**. Characterization data for compounds **15**, **16**, and **17**. HMBC correlations for **14a** and **14b**. TLC image of quinones prepared according to Scheme 2. The supplementary data are available online with the paper in ScienceDirect. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.01.049.

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27. Resin **11** (Supplementary information) R = ^{n}Pr, (250 mg, 0.35 mmol) was treated with a solution of **3** (2.5 equiv) in CH_2Cl_2 followed by addition of 2,6-di-*tert*-butylpyridine (2 equiv). The reaction mixture was protected from light and allowed to stand for 2 h then washed with CH_2Cl_2, (3×), MeOH (3×), CH_2Cl_2 (5×). Cleavage was effected by treatment with TFA–CH_2Cl_2 (1:4) (2 × 5 min). The cleavage solution was removed, combined with CH_2Cl_2 washings of the resin, evaporated under reduced pressure and analyzed by LC–MS. Chromatography on a column of neutral alumina eluting with hexane–CH_2Cl_2 gradient afforded **14a** (34 mg, 33% yield) from the more mobile band and **14b** (38 mg, 37%) from the slower eluting band. **14a** ^{1}H NMR ($CDCl_3$, 300 MHz, δ) 8.36 (1H, d, $J = 9$ Hz), 7.86 (1H, dd, $J = 9$ Hz), 7.63 (1H, d, $J = 9$ Hz), 3.80 (2H, m), 1.70 (2H, m), 1.01 (3H, t, $J = 7$ Hz). λ_{max} 486 nm, ε_{max} 3300 M^{-1} cm^{-1} (CH_2Cl_2). **14b** ^{1}H NMR ($CDCl_3$, 300 MHz, 8.22 (1H, d, $J = 9$ Hz), 7.76 (1H, dd, $J = 9$ Hz), 7.66 (1H, d, $J = 9$ Hz), 3.80 (2H, m), 1.72 (2H, m), 1.02 (3H, t, $J = 9$ Hz). λ_{max} 483 nm, ε_{max} 3400 M^{-1} cm^{-1} (CH_2Cl_2). Compounds **13**, **15**, and **16** were prepared similarly and the crude products purified by

filtration through a plug of silica gel and characterized as regioisomeric mixtures (Supplementary information). All new compounds were also characterized by HRMS.

28. More prolonged heating at higher temperatures resulted in release of **17** into solution evidently due to thermally induced cleavage. Quinone **17** has been reported previously: Kasai, T.; Kurabayaschi, Y.; Suzuki, Y.; Tsuruoka, S. *J. Synth. Org. Chem. Jpn.* **1969**, *27*, 162.
29. Mixture couplings were effected using **3** combined equivalent of carboxylic acid, HATU (3 equiv) and DIEA (3 equiv) in DMF (Ref. 5). When Fmoc-β-Ala-OH and 3-(3,4-dimethoxy-phenyl)-propionic acid were coupled in the ratio 3:1; 1:1; 1:3; and 1:99 the intensity of the bead color decreased from deep red to pink. Cleavage followed by quantification of **18** using visible spectroscopy (the extinction coefficient of pure **18** was found to be 3400 M^{-1} cm^{-1}) gave respective loadings that were consistent with the relative amounts of the carboxylic acids used in the coupling step, the last of which was ≈10 μmol/g. Thus, the sensitivity of the test is <10 μmol/g.