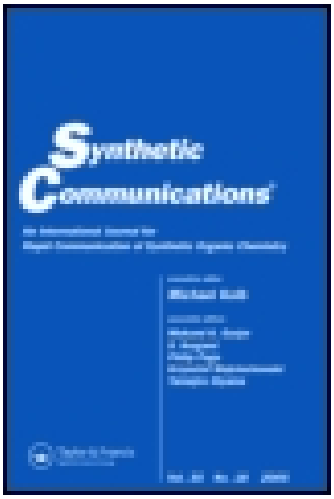

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Philip A. Helliwell [a], Ruth E. Fake [a], Kevin G. Kerr [b], Barbara Santry [a], Austen Speakman [a] & Anne Routledge [a]

[a] Department of Chemistry, University of York, Heslington, York, UK

[b] Department of Microbiology, Harrogate District Hospital, Harrogate, UK

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MICROWAVE-ASSISTED SYNTHESIS OF ARYL AMIDE BONDS ON SOLID PHASE

Philip A. Helliwell,[1] Ruth E. Fake,[1] Kevin G. Kerr,[2] Barbara Santry,[1] Austen Speakman,[1] and Anne Routledge[1]
[1]*Department of Chemistry, University of York, Heslington, York, UK*
[2]*Department of Microbiology, Harrogate District Hospital, Harrogate, UK*

A novel microwave-assisted synthesis of a library of triarylamides has been undertaken on the solid-phase.

Keywords: Arylamide; microwave; solid-phase synthesis

Compounds that promote cleavage of cellular DNA have been shown to moderate cell proliferation and as such have the potential to be used as therapeutic agents.[1] It had been demonstrated that simple triarylamide-bridged compounds (Fig. 1) could induce strand scission in DNA. The enhanced cleavage observed at pH 6.5–7.3 implied that proton transfer from aniline initiated the cleavage.[2] To probe this phenomenon, we proposed to synthesize a library of triarylamides containing a variety of functional groups (X, Y) on solid phase (Fig. 2).

The triarylamide scaffold has been used extensively as a platform for the solution-phase synthesis of mesogenic,[3] organogelating,[3] and dendrimeric[4] structures. The most common approach has utilized an amidation reaction between an acid chloride and a 3,5-diaminobenzoic ester, giving a symmetrical triarylamide scaffold. Our approach required a synthetic route that would allow us to rapidly access both symmetrical and nonsymmetrical triarylamide structures without the need to synthesize activated acid chlorides. The three nonsymmetrical triarylamide structures previously synthesized by Warner et al. in the initial study into DNA cleavage were furnished via sequential coupling (Scheme 1), which gave a moderate overall yield (28–40%) of final product.[2]

Our aim was to access a library of substituted triarylamides by performing only deprotection and coupling chemistry on the solid phase. This would be achieved by immobilizing an orthogonally protected centroid scaffold onto hydroxymethyl polystyrene and deprotecting one amine with subsequent coupling of a suitably functionalized carboxylic acid. The process would be repeated to furnish a resin-bound triarylamide (Scheme 2).

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Address correspondence to Anne Routledge, Department of Chemistry, University of York, Heslington, York YO10 5DD, UK. E-mail: ar30@york.ac.uk

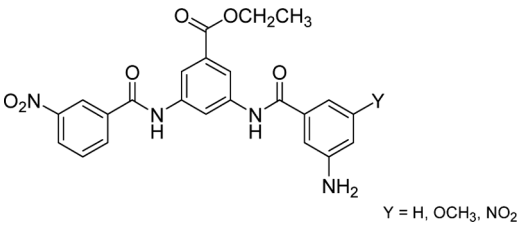


Figure 1. Triarylamide-bridged compounds inducing DNA strand scission.

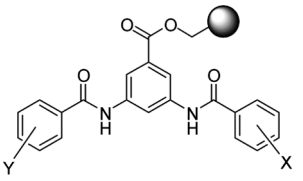
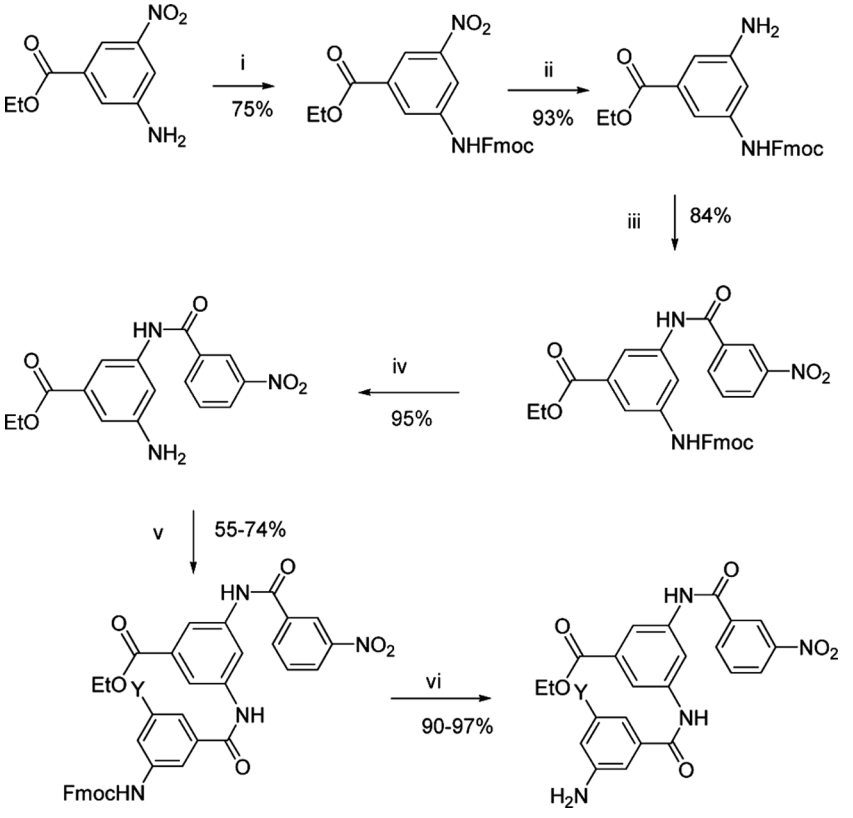
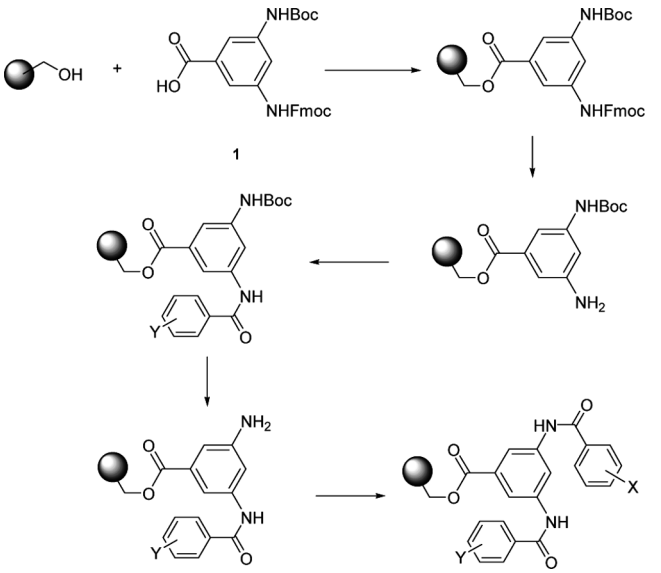


Figure 2. Proposed solid-phase library.



Scheme 1. (i) Fmoc-Cl, Na_2CO_3; (ii) cyclohexene, Pd-C; (iii) 3-NO_2-C_6H_4COCl, pyridine; (iv) piperidine, DMF; (v) PyBOP, DIPEA, DMF HOOC-Ar; (vi) piperidine, DMF.

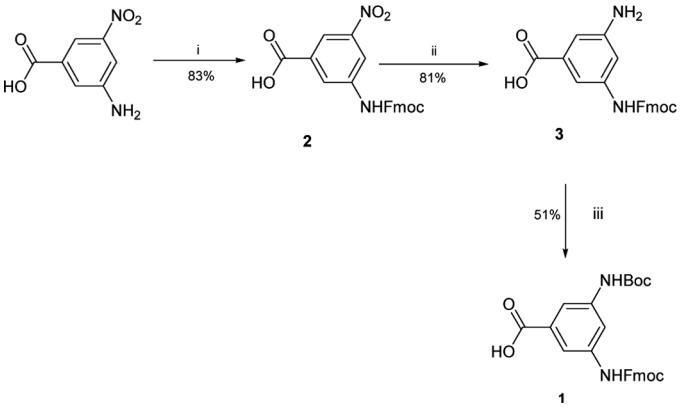


Scheme 2. Synthesis of resin-bound triarylamide library.

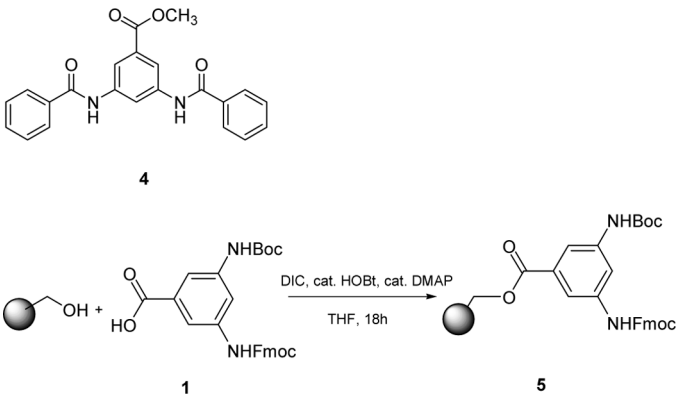
RESULTS AND DISCUSSION

The orthogonally protected centroid aromatic scaffold **1** was synthesized from commercially available 3-amino-5-nitrobenzoic acid via Fmoc protection to give **2**, with reduction to **3**[5] and subsequent Boc protection (Scheme 3).

To test the robustness of the proposed synthetic methodology for library synthesis, a simple symmetrical triarylamide **4** was the initial target molecule. Scaffold **1** was coupled to hydroxymethyl polystyrene (Advanced ChemTech, 1.1 mmol g^{-1}) using standard dicyclohexylcarbodiimide (DIC)-mediated coupling[6] (Scheme 4).



Scheme 3. (i) Fmoc chloroformate, Na_2CO_3, H_2O/dioxane (1:1), 18 h; (ii) $SnCl_2 \cdot 2H_2O$, EtOH, 70 °C, 2 h; and (iii) Boc anhydride, DIPEA, 4 h.



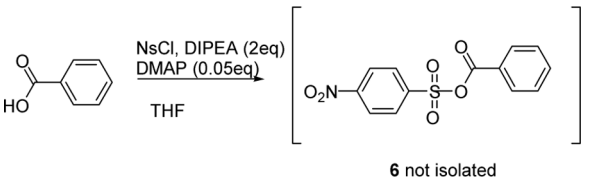
Scheme 4. Coupling of centroid scaffold **1** to hydroxymethyl polystyrene.

The coupling reaction was judged to have gone to completion by fluorescent staining of the derivatized beads **5** with *N*-methylisatoic anhydride.[7] A negative test indicated complete consumption of the resin-bound hydroxyl moiety.

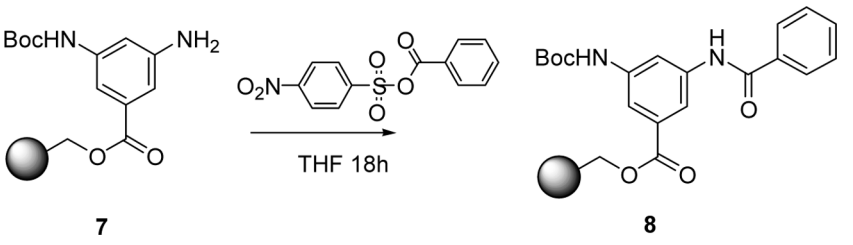
One amino group of the derivatized resin **5** was revealed using 20% piperidine in dimethylformamide[8] to remove the Fmoc protecting group. Attempts to couple the next building block (benzoic acid) using DIC-mediated coupling resulted in an incomplete reaction even after prolonged reaction times and repeated couplings. Alternative coupling reagents (HATU,[9] HBTU,[10] and EDCI[11]) failed to improve the coupling efficiency.

It has been shown that *p*-nitrobenzenesulfonyl chloride could be used to synthesize aromatic amides in solution via an activated mixed sulfonic–carboxylic anhydride.[12] Using this approach, we synthesized in situ activated building block **6** (Scheme 5) in tetrahydrofuran (THF). (The in situ solutions could be scaled up and stored at 0 °C for 24 h.)

The THF solution containing **6** was added to deprotected resin **7** (Scheme 6); the coupling was monitored by cleavage of a small portion of product resin **8** with high-performance liquid-chromatographic (HPLC) analysis of the cleavage mixture. The reaction went to completion after 18 h at ambient temperature. [Reacted resin (~5 mg) was swelled in THF/MeOH (4:1, 0.5 ml), NaOMe (5 mg) was added, and the reaction was agitated for 2 h. The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was filtered through a plug of silica and eluted with ethyl acetate (5 ml); the ethyl acetate was removed in vacuo for subsequent HPLC analysis.]



Scheme 5. In situ synthesis of activated building block **6**.



Scheme 6. On-bead formation of biarylamide.

Microwave-assisted solid-phase synthesis (MASS) is a rapidly expanding area in combinatorial synthesis[13] for both peptide[14] and small molecule[15] libraries. To explore whether the formation of the biarylamide link could be accelerated, the coupling was undertaken using microwave irradiation. Complete coupling could be achieved in minutes.

After successful attachment of the first benzoic acid group, removal of the Boc protecting group using 50% trifluoroacetic acid (TFA) in dichloromethane (DCM)[16] and microwave-accelerated coupling with **6** was repeated to furnish the resin-bound symmetrical triarylamide **9**. The triarylamide was cleaved from the resin with excess sodium methoxide in MeOH/THF (1:4) to give the methyl ester **4**. With the chemical methodology in hand, a small library of triarylamides was synthesized (Table 1).

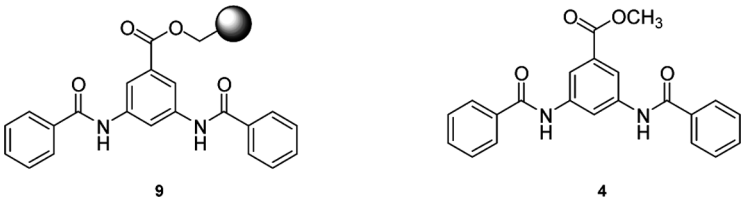


Table 1. Library of triarylamides

Compound	X[a]	Y[a]	Crude yield (%)[b]	Purity (%)[c]	Isolated yield (%)[d]
1	H	H	74	97	—
2	H	Cl	79	69	51
3	H	NO_2	72	57	39
4	H	OCH_3	62	98	—
5	NO_2	OCH_3	82	94	—
6	NO_2	NO_2	79	68	45
7	Cl	Cl	87	63	48
8	Cl	NO_2	55	30	11
9	Cl	OCH_3	86	68	42

[a]para-Substitution.
[b]Mass recovery based on the loading of resin (1.1 mmol g^{-1}).
[c]HPLC analysis of the crude product at 280 nm UV detection.
[d]Triarylamides with >90% crude purity were not purified further. Other examples were purified using PLC (2:1 ethyl acetate/petroleum ether, 40–60 °C). Isolated yields were based on the loading of resin (1.1 mmol g^{-1}).

The purity of the cleaved triarylamides proved variable. Entries 1, 4, and 5 (Table 1) gave triarylamides with excellent purity, suitable for screening. Other entries required further purification, but the overall isolated yields were comparable to those obtained by Warner[2] but were obtained in less time.

In conclusion, we have demonstrated a novel microwave-assisted solid-phase aryl amide synthesis that may find wide application in solid-phase library synthesis. The methodology was exemplified with the synthesis of a small triarylamide library. A larger library will be synthesized using the developed methodology, and DNA cleavage will be explored. The results from this study will be reported in due course.

EXPERIMENTAL

Solution-phase reagents were obtained from Aldrich Chemical Co., Fluka, or Lancaster Chemicals Ltd. Resins were supplied from Advanced ChemTech. All reagents were used as supplied unless otherwise stated. Prior to use all, resins were allowed to stand in anhydrous DCM or THF for 1 h. After each synthetic step and prior to cleavage, the solid support was exhaustively washed with a range of solvents to remove reactants from the resin matrix. A typical washing procedure used DCM (5×5 ml), DMF (5×5 ml), THF (5×5 ml), and DCM (5×5 ml). Resin washing was carried out using a commercially available vacuum manifold for parallel washing and product isolation. Solid-phase organic synthesis (SPOS) reactions were carried out in capped filtration columns of various capacities (10–25 ml) or in sealed test tubes. Reactions were agitated on a J-KEM Scientific orbital shaker (model 310). Reactions utilizing microwave techniques took place in a CEM Discover LabMate Microwave reactor in 10-ml CEM pressure vials. HPLC analysis was undertaken on a Phenomenex Prodigy 5 ODS-2 column with a Gilson 321 pump, 170 DAD, and Unipoint system software. Gradients were run using solvents (A) acetonitrile and (B) 0.1% (v/v) TFA/water, with 280-nm UV detection.

3-Amino-5-(9H-fluoren-9-ylmethoxycarbonylamino) Benzoic Acid 3

3-Nitro-5-(9H-fluoren-9-ylmethoxycarbonylamino) benzoic acid (1.61 g, 4 mmol) and tin dichloride dihydrate (4.51 g, 20 mmol) were dissolved in ethanol (60 ml) and heated under N_2 to 70 °C. After 2 h, the reaction mixture was poured onto ice, and the pH was adjusted to 7 by addition of 5% $NaHCO_3$. The reaction was extracted with ethyl acetate (3×50 ml). The organic phase was washed with brine and dried over $MgSO_4$, and the solvent was removed in vacuo to yield **3** as a yellow solid (1.20 g, 81%).

^{1}H NMR δ_H (400 MHz, DMSO-d_6): 9.62 (1H, s, N*H*), 7.91 (2H, d, *J* 8.0 Hz, Ar-*H*), 7.76 (2H, d, *J* 8.0 Hz, Ar-*H*), 7.44 (2H, t, *J* 7.5 Hz, Ar-*H*), 7.36 (2H, t, *J* 7.5 Hz, Ar-*H*), 7.25 (1H, s, Ar-*H*) 7.02 (1H, s, Ar-*H*), 6.86 (1H, s, Ar-*H*), 5.31 (2H, br s, NH_2), 4.42 (2H, d, *J* 7.0 Hz, CH_2), 4.30 (1H, t, *J* 7.0 Hz, C*H*); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6): 170.21 (COOH), 160.30 (NH-CO-O-R), 144.52 (ipso-Ar linked to COOH), 140.39 (ipso-Ar linked to NH-CO-O-R), 140.02 (ipso-Ar linked to NH_2), 136.79 (CH Ar), 134.61 (CH Ar), 128.51 (CH Ar), 127.88 (ipso-Ar of Fmoc), 127.69 (ipso-Ar of Fmoc), 124.96 (CH Ar), 120.04 (CH Ar), 116.08 (CH Ar),

81.71 (CH_2 Fmoc), 62.86 (CH Fmoc). HR-MS (ESI) $C_{22}H_{18}N_2O_4Na$ requires 397.1164; found 397.1164.

3-^{t}Butoxycarbonylamino-5-(9H-fluoren-9-ylmethoxycarbonylamino) Benzoic Acid 1

DIPEA (0.8 ml, 4.64 mmol) was added to **3** (870 mg, 2.32 mmol) in 1,4 dioxane (20 ml) and H_2O (10 ml) followed by $(Boc)_2O$ (506 mg, 2.32 mmol). The reaction was stirred at rt for 4 h. The solvent was removed in vacuo, and H_2O (20 ml) added. The pH was adjusted to 3 with 1 M HCl, the aqueous layer was extracted with EtOAc (3×50 ml), and the organic layers were washed with brine, dried over $MgSO_4$, and concentrated. Column chromatography (70:30 EtOAc/petroleum ether 40–60 °C) gave **1** as an off-white solid (554 mg, 51%). ^{1}H NMR δ_H (400 MHz, DMSO-d_6): 9.89 (1H, s, N*H*), 9.54 (1H, s, N*H*), 7.96–7.72 (7H, m, Ar-*H*), 7.46–7.34 (4H, m, Ar-*H*), 4.44 (2H, d, *J* 7.0 Hz, CH_2), 4.31 (1H, t, *J* 7.0 Hz, C*H*), 1.48 (9H, s, CH_3); ^{13}C NMR δ_C (100.6 MHz, DMSO-d_6): 180.47 (COOH), 177.16 ($2 \times$ NH-CO-O-R), 153.72 (ipso-Ar linked to COOH), 143.76 ($2 \times$ ipso-Ar linked to NH), 140.82 ($2 \times$ Ar CH), 139.96 (Ar CH), 127.90 (ipso-Ar of Fmoc), 127.28 (ipso-Ar of Fmoc), 125.72 (Ar CH), 125.61 (Ar CH), 120.36 (Ar CH), 99.72 [O-C-$(CH_3)_3$ Boc], 81.28 (CH_2 Fmoc), 46.86 (CH Fmoc), 28.37 ($3 \times CH_3$ Boc). HR-MS (ESI) $C_{27}H_{26}N_2O_6Na$ requires 497.1692; found 497.1688.

In Situ Synthesis of Benzoic 4-Nitrobenzenesulfonic Anhydride 6

Benzoic acid (61 mg, 0.5 mmol), 4-nitrobenzenesulfonyl chloride (110 mg, 0.5 mmol), dimethylaminopyridine (DMAP; 3 mg, 0.025 mmol), and diisopropylethyl amine (DIPEA; 174 µl, 1.0 mmol) were added to anhydrous THF (5 ml). The reaction mixture was agitated until no nitrobenzenesulfonyl chloride was detected by tlc (ca. 30 min.), forming **6** in situ.

Microwave-Accelerated Arylamide Synthesis

A solution of benzoic 4-nitrobenzenesulfonic anhydride **6** (1 ml) was added to deprotected resin (40–60 mg) and irradiated for 2 min (100 W, 80 °C) in a CEM 10-ml pressure vial. The resin was washed with THF (3×3 ml), and the microwave coupling was repeated.

Cleavage of Triarylamides from Resin

Reacted resin was swelled in THF/MeOH (4:1, 1 ml), NaOMe (10 mg) was added, and the reaction was agitated for 18 h. The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was filtered through a plug of silica and eluted with ethyl acetate (5 ml). The ethyl acetate was removed in vacuo to give crude triarylamide, for example, **4**. HR-MS (ESI) $C_{22}H_{19}N_2O_4$ requires 375.1339; found 375.1345.

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