Reporter Resins for Solid Phase Chemistry

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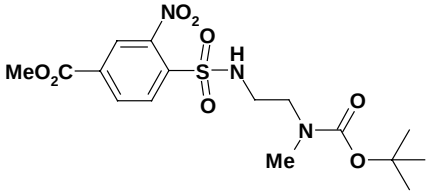
Supporting Information

General information: ^{1}H NMR spectra were recorded on a Bruker DPX 400MHz at ambient temperature in the indicated solvent. Chemical shifts are reported in parts per million (ppm) relative to chloroform (δ_H 7.24)**.** ^{1}H NMR data is reported as chemical shift, integration, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad) and coupling constant. Infrared spectra were recorded on a BioRad FTS 135 FT-IR spectrophotometer by diffuse reflectance from a KBr matrix. Analytical HPLC analysis was performed on a Hewlett Packard HP1050 fitted with a Supelcosil ABZ Plus+ 3.3cm x 4.6mm 3µm column and eluting in all cases with a mixture of the solvents H_2O/0.1%TFA (solvent A) and MeCN/0.05%TFA (solvent B) according to the following methods: Method 1 (100%A for 0.70min, then 100-0%A in B over 3.70min; 3ml/min), Method 2 (90-5%A in B over 7.00min; 1ml/min), or Method 3 (90-5%A in B over 17.0min; 1ml/min. HPLC purities were determined by the area under the curve at the stated wavelength. HPLC-MS was performed on a Micromass Platform in ESI$^+$ mode with a HP1050 front end fitted with a Supelcosil ABZ Plus+ 3.3cm x 4.6mm 3µm column eluting with either Method 1 or Method 2. Thin layer chromatography (TLC) was performed using 0.2mm aluminium backed Si 60-F plates and column chromatography was performed either over Flash Silica (Merck 9385) or using a Biotage pre-packed modular column system. All solvents and reagents were used as supplied by Fisher, Rathburn, Fluka or Aldrich unless otherwise noted. Resins were obtained from Argonaut Technologies. The following abbreviations have been used in the text: MeCN (acetonitrile), DMF (dimethylformamide), MeOH (methanol), rt (room temperature).

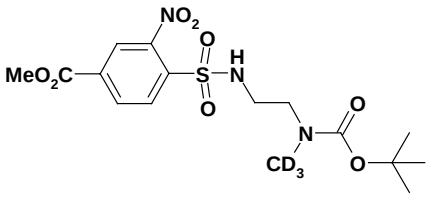
Methyl 4-[({2-[(*tert*-butoxycarbonyl)(methyl)amino]ethyl}amino)sulphonyl]-3-nitrobenzoate (3a):



A solution of the sulphonyl chloride (**2**)[7] (11.7g, 41.9mmol) in dichloromethane (160ml) was added steadily dropwise over 15mins to a stirring solution of the diamine (**1a**) (7.28g, 41.8mmol) and diisopropylamine (10.9ml, 62.7mmol) in dichloromethane (90ml) cooled in an ice-water bath. When the addition was complete the cooling bath was removed and the mixture was stirred at rt for 16h. The solvent was evaporated *in vacuo* and the residual gum was taken up in ethyl acetate (300ml) and washed with water (1x60ml), cold 1N HCl (1x60ml) and brine (1x60ml). The organic solution was dried and evaporated *in vacuo* to leave a yellow gum which was purified by column chromatography (SiO_2; Merck 9385) eluting with a mixture of ethyl acetate and hexanes (40:60) to give the *sulphonamide* (**3a**) as a viscous yellow gum (12.0g; 69%).
TLC: SiO_2: 45%EtOAc-55%hex: hυ_{254} - $KMn0_4(\Delta)$: R_f = 0.3.
HPLC: Method 2; R_t=4.97min (100%; 254nm).
LCMS: Method 2; R_t=5.04min; m/z=381.2 (MH^+-BOC).

NMR: ($CDCl_3$) δ_H 8.44(d, 1H, J=2Hz), 8.33(dd, 1H, J=2, 8Hz), 8.18(d, 1H,J=8Hz), 5.90(brs, 1H), 3.99(s, 3H), 3.37(brm, 2H), 3.29(brm, 2H), 2.84(brs, 3H), 1.44(s, 9H).

Methyl 4-[({2-[(*tert*-butoxycarbonyl)(d^3-methyl)amino]ethyl}amino)sulphonyl]-3-nitrobenzoate (3b):



A solution of the sulphonyl chloride (**2**)[7] (11.7g, 41.9mmol) in dichloromethane (70ml) was added steadily dropwise over 15mins to a stirring solution of the diamine (**1b**) (7.26g, 43.6mmol) and diisopropylamine (10.9ml, 63.1mmol) in dichloromethane (95ml) cooled in an ice-water bath. When the addition was complete the cooling bath was removed and the mixture was stirred at rt for 6h. The solvent was evaporated *in vacuo* and the residual gum was taken up in ethyl acetate (90ml) and washed with water (1x30ml), cold 1N HCl (1x30ml) and brine (1x30ml). The organic solution was dried and evaporated *in vacuo* to leave a yellow gum which was purified by column chromatography (SiO_2; Merck 9385) eluting with a mixture of ethyl acetate and hexane (45:55) to give the *sulphonamide* (**3b**) as a viscous yellow gum (11.1g; 63%).

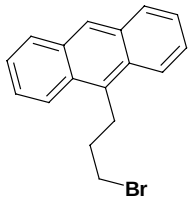
TLC: SiO_2: 45%EtOAc-55%hex: $h\upsilon_{254}$/$KMn0_4$(Δ): R_f = 0.3.

HPLC: Method 1; R_t=3.09min (100%; 254nm).

LCMS: Method 2; R_t=4.98min; m/z=419.2 (MH^+).

NMR: ($CDCl_3$) δ_H 8.44(d, 1H, J=2Hz), 8.33(dd, 1H, J=2, 8Hz), 8.20(d, 1H,J=8Hz), 5.95(brs, 1H), 3.99(s, 3H), 3.37(brm, 2H), 3.29(brm, 2H), 1.44(s, 9H).

9-(3-Bromopropyl)anthracene:



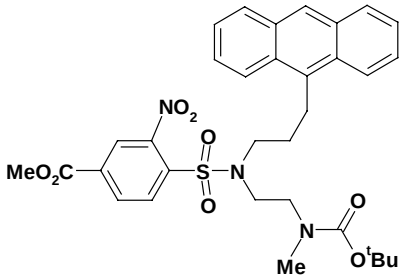
Triphenylphospine dibromide (13.9g; 33.0mmol) was added steadily to a stirring solution of anthracene-9-propanol (6.0g, 25.4mmol) and pyridine (3.3ml; 40.6mmol) in acetonitrile (50ml) and cooled to 0^oC. When the addition was complete the cooling bath was removed and the suspension was stirred at rt for 4 h. Additional triphenylphospine dibromide (1.30g, 3.30mmol) was added and stirring was continued at rt for a further 16h.. The solvent was evaporated *in vacuo* to leave a yellow solid which was washed with diethyl ether (3x60ml). The combined organic filtrates were preabsorbed onto silica gel and purified by short column chromatography eluting with a mixture of diethyl ether and hexane (20:80) to give the *bromide* as a crystalline, pale yellow solid (5.90g; 78%).

TLC: SiO_2: 10%Et_2O-90%hex: $h\upsilon_{254}$: R_f = 0.8.

HPLC: Method 1; R_t=4.13min (100%; 230nm).

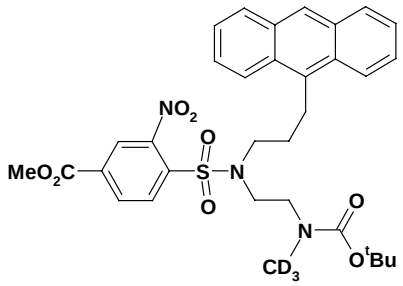
NMR: ($CDCl_3$) δ_H 8.36(s, 1H), 8.28(d, 2H, J=9Hz), 8.10(d, 2H, J=9Hz), 7.45-7.55(m, 4H), 3.79(t, 2H, J=9Hz), 3.60(t, 2H), 2.35-2.41(m, 2H).

Methyl 4-[([3-(9-anthryl)propyl]{2-[(*tert*-butoxycarbonyl)(methyl)amino]ethyl}amino)sulphonyl]-3-nitrobenzoate (4a):



2-*tert*-Butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP) on polystyrene (4.90g x 2.3mmol/g; 11.2mmol) was added steadily to a stirring solution of the sulphonamide (3a) (3.60g; 8.62mmol) and 9-(3-bromopropyl)anthracene (2.80g, 9.36mmol) in dimethylformamide (30ml) at rt. The mixture was stirred gently for 14h and then filtered to remove the resin. The resin was washed with additional dimethylformamide (3x15ml) and the combined organic filtrates were evaporated *in vacuo* to leave a yellow oil. The oil was purified by column chromatography (Biotage: KP-Sil^{TM}, 60A; 4.0x15.0cm) eluting with a mixture of ethyl acetate and hexane (20:80 then 30:70) to give the *sulphonamide* (**4a**) as a yellow foam (5.41g; 98%).
TLC: SiO_2: 40%EtOAc-60%hex: hυ_{254}: R_f = 0.3.
HPLC: Method 1; R_t=4.02min (100%; 230nm).
LCMS: Method 2; R_t=5.97min; m/z=536.4 (MH^+-BOC).
NMR: ($CDCl_3$) δ_H 8.34 (s, 1H), 8.10 (m, 1H), 8.09 (d, 2H, 9Hz), 7.98 (brd, 2H, J=9Hz), 7.97 (m, 1H), 7.75 (brm, 1H), 7.45 (m, 4H), 3.98 (s, 3H), 3.3-3.6 (brm, 8H), 2.83(brs, 3H), 1.96 (brs, 2H), 1.35-1.47 (brd, 9H).

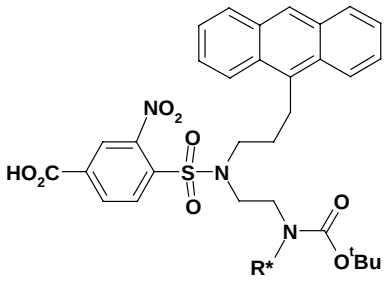
Methyl 4-[([3-(9-anthryl)propyl]{2-[(*tert*-butoxycarbonyl)(*d*^{3}-methyl)amino]ethyl}amino)sulphonyl]-3-nitrobenzoate (4b):



2-*tert*-Butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine on polystyrene (4.90g x 2.3mmol/g; 11.2mmol) was added steadily to a stirring solution of the sulphonamide (**3b**) (3.63g; 8.63mmol) and 9-(3-bromopropyl)anthracene (2.80g, 9.36mmol) in dimethylformamide (30ml) cooled in a water-bath. After 0.5h, the cooling bath was removed and the mixture was stirred gently at rt for 14h. The mixture was filtered to remove the resin and the resin was washed with dimethylformamide (3x15ml). The combined organic filtrates were evaporated *in vacuo* to leave a yellow gum which was purified by column chromatography (Biotage: KP-Sil^{TM}, 60A; 4.0x15.0cm) eluting with a mixture of ethyl acetate and hexane (20:80 then 30:70) to give the *sulphonamide* (**4b**) as a yellow foam (4.73g; 86%).
TLC: SiO_2: 40%EtOAc-60%hex: hυ_{254}: R_f = 0.3.
HPLC: Method 1; R_t=4.02min (100%; 230nm).
LCMS: Method 2; R_t=5.94min; m/z=539.4 (MH^+-BOC).
NMR: ($CDCl_3$) δ_H 8.34 (brs, 1H), 8.12 (m, 1H), 8.09 (d, 2H, J=9Hz), 7.98 (brd, 2H, J=9Hz), 7.97 (m, 1H), 7.75 (brm, 1H), 7.45 (m, 4H), 3.98 (s, 3H), 3.3-3.6 (brm, 8H), 1.96 (brs, 2H), 1.35-1.47 (brd, 9H).

[1:1] 4-[([3-(9-Anthryl)propyl]{2-[(methyl/*d*^{3}-methyl)amino]ethyl}amino)sulphonyl]-3-nitrobenzamide ArgoGel^{TM} resin trifluoroacetate (5)

[1:1] 4-[([3-(9-anthryl)propyl]{2-[(*tert*-butoxycarbonyl)(methyl/d^3-methyl)amino]ethyl} amino)sulphonyl]-3-nitrobenzoic acid



R* = CH_3/CD_3, [1:1]

The sulphonamides (**4a**) (2.40g; 3.77mmol) and (**4b**) (2.40g; 3.76mmol) were dissolved in a mixture of methanol (15ml) and tetrahydrofuran (15ml) at rt. An aqueous solution of sodium hydroxide (2N x 4.6ml; 9.2mmol) was added steadily dropwise with stirring. After stirring at rt for 3h, the mixture was acidified to ~pH5 by the addition of methanol washed Dowex H50 ion exchange resin. After 10mins, the resin was removed by filtration and washed with methanol (3x15ml). The combined organic filtrates were evaporated *in vacuo* to leave a yellow gum which was purified by column chromatography (Biotage: KP-Sil™, 60A; 4.0x15.0cm) eluting with a mixture of 5% acetic acid in ethanol and dichloromethane (5:95) to give a foam which was re-evaporated first from toluene (3 x 25ml) and then chloroform (3 x 25ml) to give the *acid* as a yellow foam (4.21g; 90%).

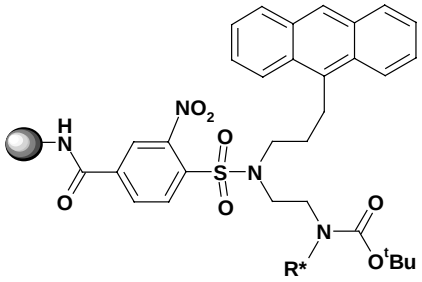
TLC: SiO_2: 5%(5%AcOH-EtOH)-95%CH_2Cl_2:hυ_{254}-$KMnO_4$(Δ) : R_f = 0.2.

HPLC: Method 1; R_t=3.92min (100%; 386nm).

LCMS: Method 2; R_t=5.94min; m/z=522.4, 525.4 (MH^+-BOC).

NMR: ($CDCl_3$) δ_H 8.33 (brs, 1H), 8.15 (brd, 1H, J=14Hz), 7.95 (d, 2H, J=9Hz), 8.00 (brm, 1H), 7.98 (brs, 2H), 7.75 (brm, 1H), 7.45 (m, 4H), 3.3-3.6 (brm, 8H), 1.98 (brs, 2H), 1.35-1.47 (brd, 9H).

[1:1] *tert*-Butyl 2-{{[4-(aminocarbonyl)-2-nitrophenyl]sulphonyl}[3-(9-anthryl)propyl] amino}ethyl(methyl/d^3-methyl)carbamate ArgoGel™ resin



R* = CH_3/CD_3, [1:1]

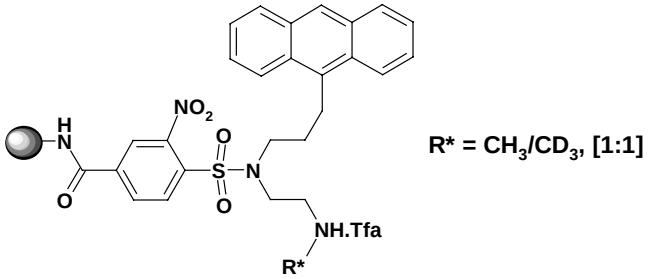
ArgoGel™ amino resin (2.00g x 0.40mmol/g; 0.80mmol) was pretreated with 30% piperidine in DMF (10ml; 1h) and then washed successively (3x10ml) with DMF, CH_2Cl_2, Et_2O, DMF and then drained. The resin was treated at rt with a solution of the above acid (750mg; 1.20mmol) and 1-hydroxybenzotriazole (324mg; 2.40mmol) in DMF (15ml). Diisopropylcarbodiimide (375µl; 2.40mmol) was added and the mixture was shaken at rt for 16h.

The resin was drained, washed successively (3x10ml) with DMF, CH_2Cl_2, Et_2O, DMF, CH_2Cl_2, Et_2O, drained and dried *in vacuo* to afford a yellow, sticky *resin* (2.48g; Kaiser test: -ve; theory loading = 0.32mmol/g).

Analytical cleavage of construct resin: (~1mg) was performed by incubating the resin with an aliquot (30µl) of a solution of mercaptoethanol (50µl) in methanolic sodium methoxide (1ml x 0.5M) at rt for 10mins. The cleavage solution was collected, diluted with (95:5) MeCN/H_2O (450µl) and analysed by HPLC-MS with detection at 386nm. A single peak was observed.

HPLC: Method 1; R_t=2.92min (100% at 386nM).
Method 3; R_t=7.60min (100% at 386nM).
LCMS: Method 2; R_t=4.45min; m/z=393.6, 396.6 (MH^+).

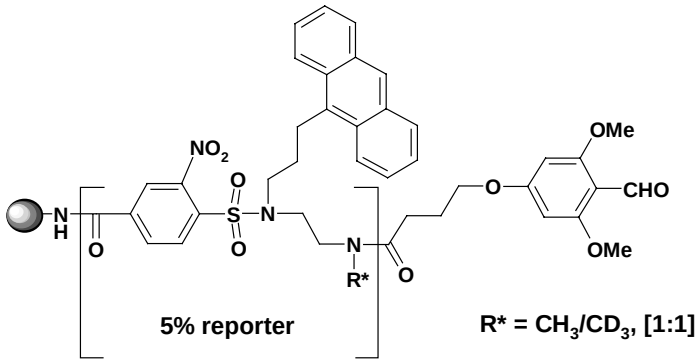
[1:1] 4-[([3-(9-Anthryl)propyl]{2-[(methyl/d^3-methyl)amino]ethyl}amino)sulphonyl]-3-nitrobenzamide ArgoGel™ resin trifluoroacetate (5)



The BOC-protected construct resin (20mg; 6µmol) was swollen with dichloromethane (0.5ml) at rt for 10min and then drained. The resin was treated twice with a mixture of trifluoroacetic acid and dichloromethane (1:1; 0.5ml) at rt for 0.5h and then drained, washed successively (3x0.5ml) with CH_2Cl_2, Et_2O, CH_2Cl_2, Et_2O. and dried *in vacuo* to afford a pale yellow resin (**5**; 19mg: Kaiser test +ve).

This material was used directly to prepare the resin (**7**).

Aldehyde resin (7) containing 5% reporter beads:



The deprotected resin (**5**) (10mg; 3µmol) was mixed with ArgoGel™ amino resin (190mg x 0.40mmol/g; 72mmol) and swollen with CH_2Cl_2 (2ml) at rt for 15mins. The mixed resin was drained and treated with a solution of 4-formyl-3,5-(dimethoxy)phenoxybutyric acid (107mg; 400µmol) and PyBOP (208mg; 400µmol) in DMF (5ml). Diisopropylethylamine (140µl; 800µmol) was added and the suspension was vigorously agitated at rt for 8h. The resin was washed successively (3x3ml) with MeCN, DMF, CH_2Cl_2, MeOH, Et_2O and dried *in vacuo* to afford a pale yellow *resin* (**7**) (~200mg: Kaiser test: -ve).

Analytical cleavage of (7): (~1mg) was performed by incubating the resin with an aliquot (30µl) of a solution of mercaptoethanol (50µl) in methanolic sodium methoxide (1ml x 0.5M) at rt for 10mins. The cleavage solution was collected, diluted with (95:5) MeCN/H_2O (60µl) and analysed by HPLC-MS with detection at 386nm. A single peak was observed.
HPLC: Method 2; R_t=4.19min (100%; 386nm).
LCMS: Method 2; R_t=2.95 min; m/z=542.7/545.7 (MH^+).

Scheme 1: Synthesis of the 'Reporter Resin'