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# Synthesis of *N*-heterocyclic ligands for use in affinity and mixed mode chromatography

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ABSTRACT

A set of heterocyclic ligands have been synthesised for use in the preparation of mixed mode affinity chromatographic adsorbents for application in the purification of proteins, including antibodies. The ligand structures were designed to consist of a pyridinyl or related aza-heterocyclic nucleus bearing a pendant arm containing either an alkylamine, alkylthiol or hydroxyalkyl nucleophilic group to allow their facile immobilisation onto an activated support matrix. Ligand diversity was achieved by altering the length of the alkyl chain between the heterocyclic nucleus and nucleophilic group, varying the position of alkyl chain attachment to the heterocycle, and incorporating extra substituents into the pyridinyl or related aza-heterocyclic ring. This diversity in ligand structure was intended to enable key structural features of the ligand, required for efficient protein binding, to be determined. In contrast to the previously used multi-step procedures for the preparation of analogous substituted pyridine or aza-heterocyclic compounds, the synthesis routes for the ligands described here have generally utilized very mild, one-step reactions with readily available heterocyclic precursors.



## 1. Introduction

Affinity and mixed mode chromatographic methods are widely utilized in the biological and biomedical sciences, e.g., for the purification of enzymes, proteins, oligo-nucleotides, DNA, including single and double coiled plasmid DNA, RNA and cells.[1–3] The technique involves the separation of the target biomolecule from other components in a complex mixture based on its ability to undergo reversible interaction with a complementary bio-specific ligand immobilised onto a support matrix. High specificity is achieved when the binding interaction replicates the same processes that occur with naturally-occurring biorecognition events. The various applications of affinity and mixed mode chromatography have been extensively discussed and reviewed in the literature.[4–8]

Commercially important classes of proteins include antibodies, which currently find wide application in therapy and diagnosis.[9,10] One of the most commonly used methods for the purification of antibodies involves affinity chromatography using immobilised protein A (a surface protein originally found over 50 years ago in the cell wall of the bacteria *Staphylococcus aureus*). However the use of protein A based affinity adsorbents has a number of well recognised limitations.[11–13] Accordingly, the search for low-molecular weight chemical ligands, which can be immobilised onto suitable support matrices and employed as a substitute for protein A in the purification of antibodies, has attracted attention over the past 20 years.

For example, Porath et al. have described a separation process for the chromatographic fractionation of protein mixtures, which they termed 'thiophilic adsorption'.[14] These researchers documented the adsorption properties of several serum proteins, including immunoglobulins (e.g., IgGs) and α2-macroglobulins, with several hydrophilic resins prepared by activation with divinylsulfone (DVS), followed by reaction with β-mercaptoethanol. This mode of adsorption is particularly affected by the presence of high concentrations of neutral kosmotropic (water structuring) salts. Subsequently, Porath and Oscarsson described a similar type of binding behaviour with some proteins when 2-mercaptopyridine was coupled to an epichlorohydrin (epoxy-) activated gel (Fig. 1).[15] Although the immobilised 2-mercaptopyridine ligand was found to

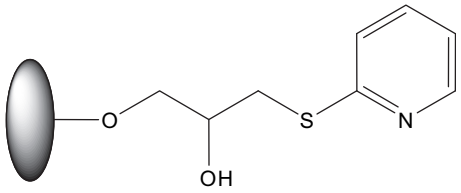


**Fig. 1.** 2-Mercaptopyridine coupled to an epichlorohydrin-activated gel.

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adsorb a greater amount of IgG when compared to the 4-substituted derivative at comparable immobilisation densities, the latter was more selective.[16] Moreover, when the 2-mercaptopyridine ligand was replaced by 2-aminopyridine or 2-hydroxypyridine significant reduction in adsorption capacity was observed, clearly indicating the need for a sulfur atom to be present.

Similarly, Knudsen et al. have prepared a variety of thiophilic adsorbents based on the immobilisation of heteroaromatic ligands onto divinylsulfone-activated gels.[17] For example, isolation of IgG was achieved from human serum in the presence of lyotropic salts using affinity adsorbents derived from 2-, 3- or 4-hydroxypyridine, 2-aminopyridine, 4-aminobenzoic acid, 4-methoxyphenol and imidazole.[17] Extending the work of Porath and Oscarsson[14], Schwarz et al. immobilised 2-mercaptopyridine, 2-mercaptopyrimidine and mercapto-thiazoline onto both epoxy-activated agarose and silica.[18] These latter two ligands were chosen on the basis of their higher hydrophilicity and a higher electron density compared to 2-mercaptopyridine. Schwarz later prepared several adsorbents using five-membered heterocyclic rings containing at least two heteroatoms[19] whilst Scholz et al. coupled 2-mercaptopyridine, 2-mercaptopyrimidine and 2-mercaptonicotinic acid to DVS- and epichlorohydrin-activated Sepharose.[20]

When compared to protein A affinity chromatography, these thiophilic chromatographic materials potentially have a number of advantages, including: (i) lower cost to produce the adsorbent; (ii) broader specificity for binding to antibodies and other proteins present in various feedstocks; (iii) the possibility to realize higher binding capacities if high density immobilisation could be achieved; (iv) milder elution conditions; (v) greater chemical stability of the immobilised ligands, (vi) possibility for reduced ligand leakage under alkaline clean-in-place (CIP) conditions and (vii) ability to separate and purify antibody fragments that lack a protein A biorecognition site.

A further extension of the use of heteroaromatic ligands immobilised onto support materials can be found in hydrophobic charge induction chromatography (HCIC). This technique, first described by Burton and Harding,[21] takes advantage of the pH-dependent behaviour of ligands, such as 4-mercaptoethylpyridine (4-MEP). This ligand has a greater specificity for immunoglobulins than 4-mercaptoethylbenzene.[22] In previous studies, this ligand has been chemically attached to a hydrophilic cellulose-based resin which has been previously activated with allyl bromide. The resulting adsorbent, MEP HyperCel®, is commercially available. Boschetti and Guerrier[23] have prepared a number of MEP-related ligands including ones where the sulfur atom was replaced by an oxygen or nitrogen atom. These alternatives, however, showed minimal adsorption of IgG, again indicating the importance of the presence of the sulfur atom.

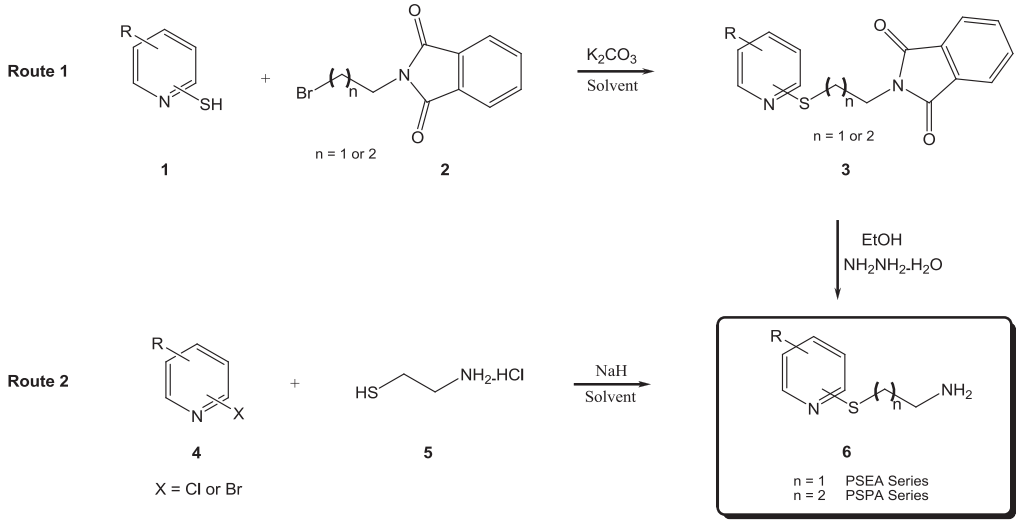
With the ever increasing need for more efficient and cost effective protocols for the purification of therapeutic monoclonal antibodies, considerable motivation exists[24] to develop other more effective low-molecular weight molecules that are able to: (i) bind antibodies with similar affinity to protein A and (ii) possess improved physical and chemical properties. Moreover, these synthetic ligands should preferentially have characteristics that result in increased resistance to degradation from chemical and biological agents, lower toxicity, reduced leakage, high antibody binding capacities and broader selectivity. Overall, improvements in the design and synthesis of new molecules with these properties will lead to significantly reduced production costs for antibodies, particularly therapeutic monoclonal antibodies.

Herein, we report the synthesis of a range of low-molecular weight ligands based on several *N*-heterocycles containing a primary alkylamine group. Our synthetic objective was to prepare these compounds in high purity by efficient methods and to subsequently immobilise them onto suitable solid phase support materials. Our preliminary results with these new affinity adsorbents indicate their potential for the static (batch) capture and chromatographic purification of antibodies.

## 2. Results and discussion

The compounds prepared in this work retain a heterocyclic core and exocyclic sulfur atom but in contrast to the ligands developed earlier by Oscarsson and Porath[16] or other investigators, the novel ligands described here utilize an aliphatic primary amine tether, which enables both the very efficient immobilisation of the ligand onto a solid support material and simultaneously provides a spacer group between the interactive core and the solid support. In this manner, this pendant structure is attached to the heterocyclic ring via the exocyclic sulfur atom and can be readily varied in structure to allow the hydrophobicity of the ligand to be easily altered.

For example, the PSEA and PSPA series (**6**, Scheme 1) possess an ethyl- or propyl-amine group extending from the exocyclic sulfur, respectively. These derivatives were prepared by hydrazinolysis of the corresponding phthalimides, based on modifications to the procedures for the synthesis of *N*-alkylthiopyridine-benzo[*b*]thiophene-2-carboxamides or 4-(2-pyridylthio)butylamino-methylenebisphosphonate and other amino-methylene-bisphosphonic

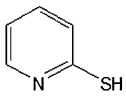
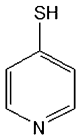
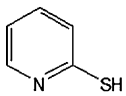
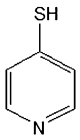



**Scheme 1.** General route for the preparation of the pyridinylsulfanylethyl- and propyl-amine compounds, **6**.

acid derivatives described by Boschelli et al.[25] and Sodha et al.,[26] respectively (route 1, Scheme 1).

The 2- and 4-mercaptopyridines **1** were coupled to *N*-(2-bromoethyl)phthalimide **2**, $n$=1 or *N*-(2-bromopropyl)phthalimide **2**, $n$=2 in DMF or refluxing acetone in the presence of potassium carbonate. The phthalimides **3** were purified by trituration, recrystallisation or flash chromatography and obtained in moderate yields (Table 1). The phthalimides **3** were converted into the corresponding primary amines **6** by the use of hydrazine hydrate in EtOH. The desired amines **6** were obtained in reasonable yields (Table 1) after a basic (pH ~9) work-up, with only the 4-PSPA **6d** requiring further purification. All of the synthesized compounds were characterised by $^{1}$H and $^{13}$C NMR, FTIR, MS, and melting point and by high resolution electrospray mass spectrometry and elemental analysis for all new compounds. Since the two-step synthesis described above led to only ca. 40% overall yield for both and 2-aminoethanethiol hydrochloride **5** modelled on the method originally described for the synthesis of 2-[[2-(dimethylamino)ethyl]thio]-3-phenylquinoline by Blackburn et al.[30] The 2-PSEA derivative **6a** was obtained in 71% yield while the 4-PSEA derivative **6b** was acquired in an 87% yield (Table 2, entries i and ii). These yields are superior to the <40% yield for the same derivatives prepared by the earlier two step procedures[31] of route 1. Although the purification of 4-pyridinylsulfanylethylamine, **6b**, prepared via route 2, was attempted by distillation under high vacuum, this resulted in decomposition of the crude product and formation of two unidentified compounds. However, the target 4-PSEA, **6b**, could be readily purified in very high isolated yield (87%) by column chromatography using silica Si60 and dichloromethane/methanol/ammonium hydroxide (9:1:0.1, v/v) as the eluent. Similar chromatographic methods were also used with other substituted pyridinylsulfanylethylamines in circumstances where attempted distillation lead to decomposition.

**Table 1**
Preparation of pyridinyl sulfanyl ethylamines **6** (R=H) via route 1 (Scheme 1)

| Entry | **6** | **1** | **2** | Step 1 | | | | Step 2 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Solvent | Temp | Time | Yield % **3** | Time | Yield % **6** |
| i | **6a**; 2-PSEA |  | $n$=1 | Acetone | Reflux | 2 h | 64 | 16 h | 58 |
| ii | **6b**; 4-PSEA |  | $n$=1 | Acetone | Reflux | 2 h | 43 | 16 h | 83 |
| iii | **6c**: 2-PSPA |  | $n$=2 | Acetone | Reflux | 4 h | 94 | 17 h | 96 |
| iv | **6d**; 4-PSPA |  | $n$=2 | DMF | rt | 3.5 h | 48 | 16 h | 68 |

Specific methods for the synthesis of these compounds can be found in the Experimental section. Literature data for known compounds can be found at the following citations: **3a**;[26,27] **3b**;[26,28] **3c**;[26] **3d**;[25] **6a**;[29] **6b**;[26] **6c**;[26] **6d**.[26]

PSEA derivatives (Table 1, entries i and ii), alternative methods were sought.

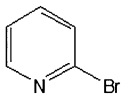
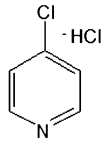
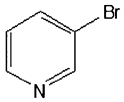
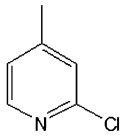
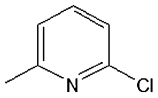
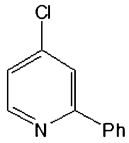
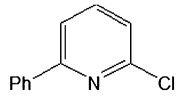
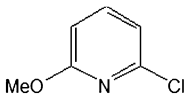
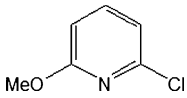
The preparation of 2-PSEA has previously been reported in a 77% yield by Constable et al.,[29] whereby 2-bromopyridine was allowed to react with 2-aminoethanethiol hydrochloride **5** in a solution of sodium ethoxide in EtOH at reflux for 24 h. The method investigated here (route 2, Scheme 1) also involves the reaction of a halogenated pyridine and **5**, however in this case the reaction proceeded more rapidly under much milder conditions using NaH in dry THF and/or hexamethylphosphoramide (HMPA) or DMF at rt (Table 2). These conditions removed the possibility of the ethoxide base acting as a nucleophile as observed earlier by Constable et al.[29] or by us during the preparation of 4-QSEA (see later discussion). Furthermore, compared to route 1, route 2 also has the benefit that a larger range of substituted halogenated pyridines are more readily available compared to the corresponding substituted mercapto-pyridines, allowing for more versatile preparation of a diverse library of derivatives with varying properties, e.g., ligands with different hydrophobicity, steric bulk and electron-withdrawing or electron-donating effects.

The ethylamines **6** were therefore synthesised in a one-step, one pot reaction from commercially available halogenated pyridines **4**

Preparation of 3-PSEA **6e** via route 2 led to a 58% conversion of the starting 3-bromopyridine to the product and an isolated yield of 50% after purification by flash chromatography (Table 2, entry iii). Alternatively, the preparation of 3-PSEA **6e** was achieved by firstly converting the starting 3-bromopyridine to the *N*-oxide derivative in order to facilitate nucleophilic attack at the 3-position of the ring. This was achieved by the oxidation of 3-bromopyridine, based on the oxidation of 2-bromopyridine with hydrogen peroxide in glacial acetic acid as described by Evans and Brown[32] and Ochiai,[33] giving the *N*-oxide in a 70% yield without the need for purification. 3-PSEA was then prepared in a 38% yield by reacting 3-bromopyridine *N*-oxide with 2-aminoethanethiol hydrochloride **5** followed by addition of phosphorus trichloride based on a procedure for the preparation of 3-arylthio-pyridones described by Batmanghelich and Turner.[34]

For synthesis of these compounds via route 2 (Scheme 1) it was observed the yield was enhanced when a slurry of NaH in neat HMPA was used, i.e., without the addition of THF. The preparation of the Me-2-PSEA derivatives was achieved (via route 2, Scheme 1) in acceptable yields of 38% and 63% for the 4-Me and 6-Me analogues **6f** and **6g**, respectively, after work-up and purification (Table 2, entries vi and v). Preparation of Me-2-PSEA derivatives was initially attempted following the method described by Constable et al.;[29] however, under

**Table 2**
Preparation of pyridinyl sulfanyl ethylamines **6** (R=H, Me, Ph, OMe) via route 2 (Scheme 1)

| Entry | 6 | 4 | Solvent | Temp | Time | Yield % **6** |
|---|---|---|---|---|---|---|
| i | **6a**; 2-PSEA |  | THF/HMPA | rt | 22 h | 71 |
| ii | **6b**; 4-PSEA |  | THF/HMPA | rt | 2 days | 87 |
| iii | **6e**; 3-PSEA |  | HMPA | rt | 2 days | 50 |
| iv | **6f**; 4-Me–2-PSEA |  | THF/HMPA | rt | 2 days | 38 |
| v | **6g**; 6-Me–2-PSEA |  | HMPA | rt | 22 h | 63 |
| vi | **6h**; 2-Ph–4-PSEA |  | HMPA | rt | 3 h | 82 |
| vii | **6i**; 6-Ph–2-PSEA |  | HMPA | rt | 27 h | 82 |
| viii | **6j**; 6-OMe–2-PSEA |  | HMPA | rt | 2 days | 30 |
| ix | **6j**; 6-OMe–2-PSEA |  | DMF | 60 °C | 23 h | 42 |

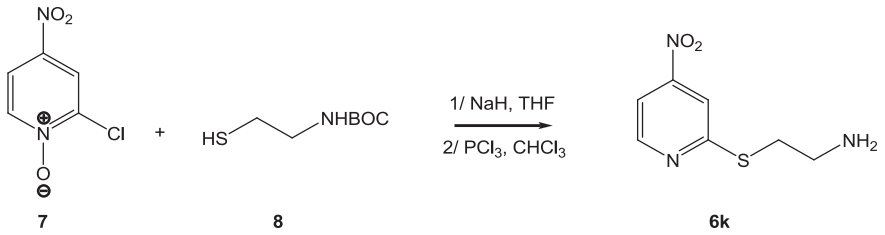
Specific methods for the synthesis of these compounds can be found in the Experimental section. Literature data for known compounds can be found at the following citations: **6a**;[29] **6b**,[26] **6e**.[31]

these conditions the $^1$H NMR spectra of the reaction mixture showed only ca. 10% conversion to the desired product in each case.

To prepare the desired phenyl derivatives, commercially available 2-phenylpyridine was chlorinated at the α- and γ-positions of the pyridine ring, again via the *N*-oxide, and obtained in a 72% yield by a procedure modelled on the synthesis by Zhang et al.[35] of the 4-pyridyl substituted tri-dentate bis[2-(2-pyridyl)ethyl]methylamine ligands. Chlorination and subsequent reduction of the *N*-oxide was achieved via a method similar to that described for the preparation of furo[2,3-*b*/*c*]pyridine *N*-oxides by Shiotani and Taniguchi.[36] Purification by column chromatography of the reaction mixture afforded the 2-chloro-6-phenylpyridine (52%), 4-chloro-2-phenylpyridine (24%) and 5-chloro-2-phenylpyridine (0.8%). The 2- and 4-chloro products were then reacted with 2-aminoethanethiol hydrochloride **5** (route 2, Scheme 1), with both of the phenyl derivatives **6h** and **6i** obtained in 82% yield after chromatography (Table 2, entries vi and vii).

The electron-donating effect of the methoxyl group decreased the reactivity of the *meta*-positioned chlorine substituent, resulting in longer reaction times when compared to the 6-Me-2-PSEA derivative **6g** under the same conditions. The amine hydrochloride salt of 6-OMe-2-PSEA **6j** was prepared in a 39% yield by the addition of a saturated solution of HCl in ether. Basification afforded the free amine in a 30% overall yield (Table 2, entry viii). This yield was increased to 42% when the reaction was performed in DMF at 60 °C (Table 2, entry ix).

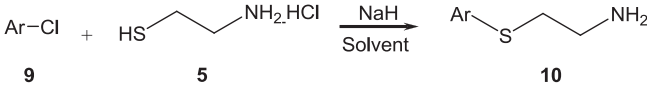
Electron withdrawing substituents positioned *meta* with respect to the nucleophilic substitution position do not participate in resonance stabilisation of the intermediate and therefore do not assist substitution. Preparation of 4-$NO_2$-2-PSEA **6k** was, however, achieved via the *N*-oxide **7** in a one-pot synthesis (Scheme 2). The starting chloro-pyridine *N*-oxide **7** was reacted with the carbamate **8** in a suspension of NaH in THF before deprotection of the BOC group and reduction of the *N*-oxide with a solution of $PCl_3$ in $CHCl_3$. The target 4-$NO_2$-2-PSEA **6k** was obtained in ca. 68% yield and 95% purity. A sample of the product was purified by preparative TLC (silica Si60) for characterisation. In order to minimise the possible displacement of the 4-nitro group as well as the 2-chloro group, a slight excess of the pyridine *N*-oxide **7** was used in respect to the carbamate **8**.




**Scheme 2.** Preparation of 4-$NO_2$–2-PSEA.

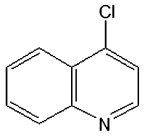
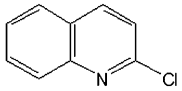
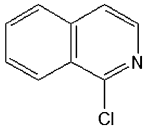
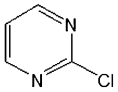
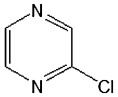
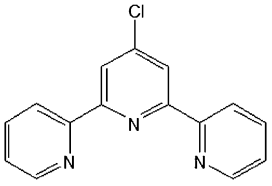
Preparation of 4-$NO_2$-2-PSEA **6k** was originally attempted using a procedure based on route 2 (Scheme 1). The product was isolated as the HCl salt and the free amine regenerated to give a 36% yield of 2-Cl-4-PSEA rather than 4-$NO_2$-2-PSEA, i.e., substitution of the $NO_2$ group occurred in preference to the chloro group. This result is consistent with other literature precedents, and indicated that nucleophilic attack would be preferentially favoured at the carbon bearing the halogen (α-position) compared to the carbon bearing the nitro group (γ-position), if the electronic properties of the pyridyl ring were firstly converted to the *N*-oxide derivative.[37]

Based on the above synthetic methodologies, a range of pyridinylsulfanylethylamines **6** have been prepared under mild conditions from readily available halogenated pyridines **4**. The generally good yields obtained make this route (route 2, Scheme 1) preferable when compared to the alternative two step route from pyridine thiols **1** (route 1, Scheme 1). The one pot route to the 4-nitro compound **6k** (Scheme 2) illustrates the utility of *N*-oxides in this mode of nucleophilic substitution chemistry with strongly electron-withdrawing substituents. Moreover, the route employed above for preparation of various pyridine derivatives **6** (route 2, Scheme 1) can be utilized to prepare related compounds from other heteroaryl chlorides. Thus, a range of different heteroaryl sulfanyl ethylamines **10** were prepared from readily available heteroaryl chlorides as shown in Scheme 3 with the results summarized in Table 3.




**Scheme 3.** General route for preparation of sulfanyl ethylamines **10**.

**Table 3**
Preparation of heteroaryl sulfanyl ethylamines **10** via Scheme 3

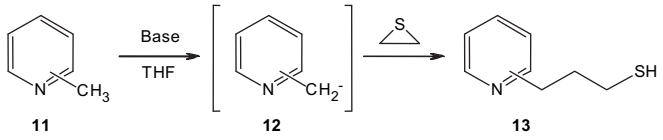
| Entry | **10** | **9** | Solvent | Temp | Time | Yield % **10** |
|---|---|---|---|---|---|---|
| i | **10a**; 4-QSEA |  | HMPA | rt | 1 h | 52 |
| ii | **10b**; 2-QSEA |  | HMPA | rt | 2 h | 84 |
| iii | **10c**; 1-IQSEA |  | HMPA | rt | 2.5 h | 91 |
| iv | **10d**; 2-PymSEA |  | HMPA | rt | 3 h | 70 |
| v | **10e**; 2-PyzSEA |  | HMPA | rt | 3 h | 85 |
| vi | **10f**; 4-TerPSEA |  | DMF | 50 °C | 3 h | 95 |

For example, the 4-quinoline derivative (4-QSEA, **10a**) was prepared as described in Scheme 3 and isolated as the amine salt (71%) before conversion to the free base (52%) (Table 3, entry i). Purification of the free base was attempted by vacuum distillation, however the product decomposed on heating. An alternative method for the preparation of this compound modelled on the procedure of Constable et al.[29] for the synthesis of 2-PSEA with

ethanolic sodium ethoxide gave complete conversion of the starting material into a 60:40 ratio of 4-QSEA and 4-ethoxyquinoline. Separation by column chromatography led to the isolation of 4-ethoxyquinoline (30%) but failed to yield 4-QSEA in a high purity state. Subsequent trituration of the impure 4-QSEA fraction facilitated easy isolation of a new product *N*-[2-(quinolin-4-ylsulfanyl)-ethyl]-quinolin-4-amine (11%). The 2-quinoline derivative (2-QSEA, **10b**) and the 1-*iso*-quinoline derivative (1-IQSEA, **10c**) were also prepared as one pot reactions by the method described in Scheme 3. Both products were purified by precipitation of the amine salt with subsequent conversion to the free base affording excellent yields of 2-QSEA and 1-IQSEA (84% and 91%, respectively) (Table 3, entries ii and iii). A small sample of 2-QSEA was also purified by column chromatography and found to be stable, unlike 4-QSEA.

The preparation of 2-PymSEA **10d** was achieved in 70% yield after purification by column chromatography while 2-PyzSEA **10e** was obtained in 85% yield (Table 3, entries iv and v). Similarly, 4-TerPSEA[38] **10f** was prepared via the method outlined in Scheme 3 using DMF as the solvent and obtained in a 95% yield with no column chromatographic purification required. The starting 4′-chloro-2,2′:6′,2″-terpyridine derivative was prepared as described by Constable and Ward[39] in an overall yield of 52% from ethyl pyridine-2-carboxylate.

The preparation of propanethiol pyridines (MPP series) has previously been described by Furukawa et al.[40] and involved conversion of commercially available hydroxyl derivatives into the chloro derivatives and finally to the desired thiols. An alternative one-step synthesis was investigated here and involved the reaction of ethylene sulfide (thiirane) and the desired picoline **11** (Scheme 4). This synthesis was achieved based on a method described for the preparation of 2,2′-hydroxymethyl-substituted 4,4′-bipyridines by Leighton and Sanders, who employed ethylene oxide to prepare the corresponding propyl alcohol derivative.[41]

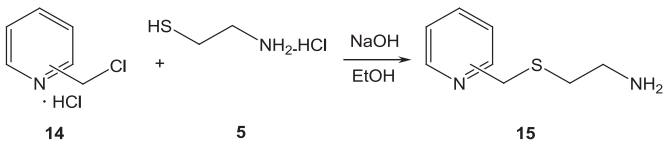



**Scheme 4.** General route for the preparation MPP derivatives.

The thiols were prepared by firstly generating the anion **12** of the starting picoline **11** with either butyllithium or lithium diisopropylamide (LDA), a more hindered base, and then adding thiirane at −78 °C. The results are summarized in Table 4. The 2-MPP **13a** was obtained in a 45% yield after purification by distillation (Table 4, entry i). The crude product obtained for the 4-derivative was distilled to give a side product, 2-*n*-butyl-4-methylpyridine and 4-MPP **13b**, which was obtained in a 10% yield after column chromatography (Table 4, entry ii). In addition, the disulfide dimer, 4,4′-(disulfanediyldipropane-3,1-diyl)di-pyridine, formed by oxidation of 4-MPP during the chromatographic step, was also isolated (4%).

Lithium diisopropylamide (LDA) was also used in the preparation of the 3-derivative to prevent nucleophilic attack of the butyl group, which had occurred during the preparation of 4-MPP. A low yield of 3% was obtained for 3-MPP (Table 4, entry iii) due to incomplete conversion of the starting materials and formation of multiple side products, which necessitated a two step purification process involving distillation and column chromatography. Although the one pot route utilizing thiirane gave low yields of the 3- and 4-MPP, the new 2-isomer **13a** was obtained in 45% yield. This yield compares favourably to the ca. 40% yield obtained for the two step preparation of 3- and 4-MPP using $H_2S$ as described by Furukawa et al.[40]

The PMSEA derivatives **15** were prepared based on methods used by Tilley et al.[42] for the synthesis of *N*-(heterocyclic alkyl)pyrido[2,1-*b*]quinazoline-8-carboxamides or Carson et al.[43] for the synthesis of (*E*)-3-(4-oxo-4*H*-quinazolin-3-yl)-2-propenamides, as shown in Scheme 5. The desired picolyl chloride hydrochloride **14** was reacted with 2-aminoethanethiol hydrochloride **5** in a solution of NaOH in ethanol. The crude products were purified by column chromatography to give very good yields of all three isomers (Table 5, entries i–iii).




**Scheme 5.** General route for the preparation of PMSEA derivatives.

**Table 4**
Preparation of the MPP derivatives **13**

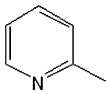
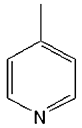
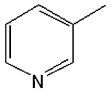
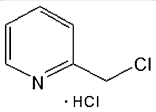
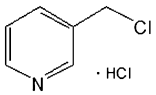
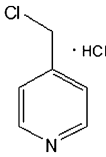
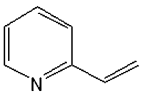
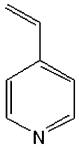
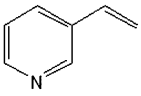
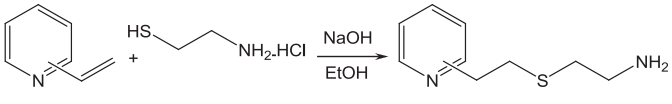
| Entry | **13** | **11** | Solvent | Temp | Time | Base | Yield % **13** |
|---|---|---|---|---|---|---|---|
| i | **13a**; 2-MPP |  | THF | rt | o/n | *n*-BuLi | 45 |
| ii | **13b**; 4-MPP |  | THF | rt | o/n | *n*-BuLi | 10 |
| iii | **13c**; 3-MPP |  | THF | rt | o/n | LDA | 3 |

**Table 5**
Preparation of PMSEA **15** and PESEA **17** derivatives

| Entry | **15** or **17** | **14** or **16** | Solvent | Temp | Time | Yield % **15** or **17** |
|---|---|---|---|---|---|---|
| i | **15a**; 2-PMSEA |  | EtOH | rt | 2.5 h | 89 |
| ii | **15b**; 3-PMSEA |  | EtOH | rt | 2.5 h | 75 |
| iii | **15c**; 4-PMSEA |  | EtOH | rt | 2.5 h | 94 |
| iv | **17a**; 2-PESEA |  | EtOH | Reflux | 2.5 h | 95 |
| v | **17b**; 4-PESEA |  | EtOH | Reflux | 2.5 h | 82 |
| vi | **17c**; 3-PESEA |  | EtOH | Reflux | 2.5 h | 0 |

Specific methods for the synthesis of these compounds can be found in the Experimental section. Literature data for known compounds can be found at the following citations: **15a**;[47] **15b**;[42] **15c**;[43] **17a**.[44]

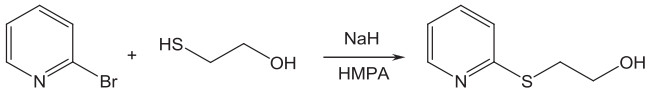
The PESEA derivatives **17** were synthesised based on a method described by Kaasjager et al.[44] for the preparation of 1-(2′-pyridyl)-3-thia-5-aminopentane as shown in Scheme 6. The desired vinylpyridine **16** was reacted with 2-aminoethanethiol hydrochloride **5** in a solution of NaOH in ethanol to give good yields of the 2- and 4-derivatives (Table 5, entries iv and v). Only the 4-derivative **17b** required purification (by column chromatography) to remove the starting compound, 4-vinylpyridine. While the 2- and 4-vinylpyridines were commercially available, 3-vinylpyridine was prepared in 32% yield, based on a procedure described by Alunni et al.[45] for the preparation of isomeric 2-fluoroethyl-pyridines, involving a Wittig reaction of 3-pyridinecarboxyaldehyde with methyltriphenylphosphonium bromide. The preparation of 3-PESEA **17c** using the method described above was not successful, possibly due to the lack of resonance stabilisation of an intermediate formed following attack of the thiol at the β-carbon of the 3-substituted pyridine ring.




16 5 17

**Scheme 6.** General route for the preparation of PESEA derivatives.

The final compound prepared to complete the ligand set was 2-PSEOH **19**, the alcohol terminated analogue of the 2-PSEA derivative **6a**, which contains a terminal primary amine. The alcohol was prepared by reacting 2-mercaptoethanol **18** and 2-bromopyridine in a slurry of NaH and HMPA at 40 °C (Scheme 7). An alternative strategy to achieve the $S_N1$ nucleophilic displacement of the halo-atom by a sulfur anion has been employed by Beugelmans et al. for the preparation of benzothiophenes and thienopyridines.[46] Purification of the crude product by column chromatography gave the alcohol **19** in ca. 72% yield (and ca. 95% purity). A similar reaction at ambient temperature gave only 54% conversion.




18 19

**Scheme 7.** Preparation of 2-PSEOH.

Based on the above synthetic procedures, a variety of *N*-heterocyclic sulphanyl compounds with alkyl side chains bearing a terminal primary amine, alcohol or thiol group were thus prepared. Changes to the ligand structure and properties were achieved by including various ring substituents. In associated experiments, these compounds were immobilised, using epichlorohydrin-activated Sepharose Fast Flow® as the model solid phase support material with the objective to evaluate their potential as novel mixed mode affinity chromatographic adsorbents for the binding and purification of monoclonal antibodies (mAbs) in both static (batch) or dynamic (column) modes.

The choice of epichlorohydrin for the activation of the agarose-based Sepharose Fast Flow® was based on the well documented observation[48,49] that stable O–C bonds are generated during the activation stage with polysaccharide-related polymers rich in hydroxyl groups, such as agarose or cellulose, whilst similarly stable C–N bonds are formed following the nucleophilic opening of the introduced epoxide functionality by the amino group of the introduced ligand. As a consequence, adsorbents derived from these epichlorohydrin-activated gels can be re-used for multiple cycles without significant loss of performance provided feedstock fouling due to host cell components is not significant. In the present studies this favourable attribute was manifested, when the adsorbents were recycled, showing no significant loss of the immobilised ligand for at least 10 cycles following batch regeneration with 0.5 M NaOH. As shown in Tables 6 and 7 several of these resins (selected on the basis of their ligand density and the magnitude of their $Q_m$ values) could be obtained with ligand densities in the range of 282–346 μmol/g adsorbent as determined by nitrogen elemental analysis. Immobilised ligand densities in this range were anticipated to result in high static $Q_{max}$ values (as mg mAb bound per mL), indicative of high capacities for mAbs and suitability for dynamic chromatographic use. In comparison, the remaining immobilised ligands either showed lower binding efficiencies or lower overall recovery. With regard to the five exemplars shown in Tables 6 and 7, regeneration studies showed that these adsorbents were relatively stable to clean-in-place (CIP) treatments with 0.5 M NaOH during the regeneration stages whilst high mAb binding capacities were retained when employed in the dynamic recycling/re-use chromatographic mode of operation.

**Table 6**
Static binding capacity of several adsorbents, following immobilisation of the ligand onto epichlorohydrin-activated Sepharose 6 Fast Flow (Seph–). The $Q_{max}$ and $R^2$ values were derived from the fit of the experimental data to a first order Langmuir isotherm

| Adsorbent | Ligand Density μmol/g | $Q_{max,\ static}$ mAb Bound mg/mL | $R^2$ (Langmuir Isotherm) |
|---|---|---|---|
| Seph–2-QSEA | 329 | 55.6 | 0.990 |
| Seph–4-$NO_2$–2-PSEA | 307 | 52.8 | 0.981 |
| Seph–2-PSEA | 307 | 24.7 | 0.988 |
| Seph–2-PSPA | 282 | 28.0 | 0.966 |
| Seph–4-QSEA | 346 | 55.3 | 0.990 |

**Table 7**
Dynamic binding capacity of several adsorbents for the mAb, following immobilisation of the corresponding ligand onto epichlorohydrin-activated Sepharose 6 Fast Flow (Seph–), and the corresponding mAb recovery from the eluted peak

| Adsorbent | Ligand Density μmol/g | $Q_{max,\ dynamic}$ mAb Bound mg/mL | Recovery (%) |
|---|---|---|---|
| Seph–2-QSEA | 329 | 26.9 | 87 |
| Seph–4-$NO_2$–2-PSEA | 307 | 52.8 | 91 |
| Seph–2-PSEA | 307 | 24.7 | 90 |
| Seph–2-PSPA | 282 | 28.0 | 91 |
| Seph–4-QSEA | 346 | 55.3 | 90 |

Moreover, as evident from Fig. 2, when used in the chromatographic mode efficient and selective capture, elution and column regeneration could be achieved for the purification of the target antibody.

As anticipated, the maximum binding capacities determined by static binding experiments were greater that the binding capacity achieved under the working conditions of a dynamic chromatographic separation. Nevertheless, these dynamic binding capacities (25–52 mg/mL) compare very favourably with other mAb binding chromatographic adsorbents, such as protein A Sepharose Fast Flow® (15–20 mg/mL)[50] or MEP HyperCel® (30–35 mg/mL).[51] As such, these studies indicate that this new class of heterocyclic ligand may have significant potential in the purification of mAbs and possibly other proteins. To this end, further investigations are underway documenting this potential with feedstocks of greater

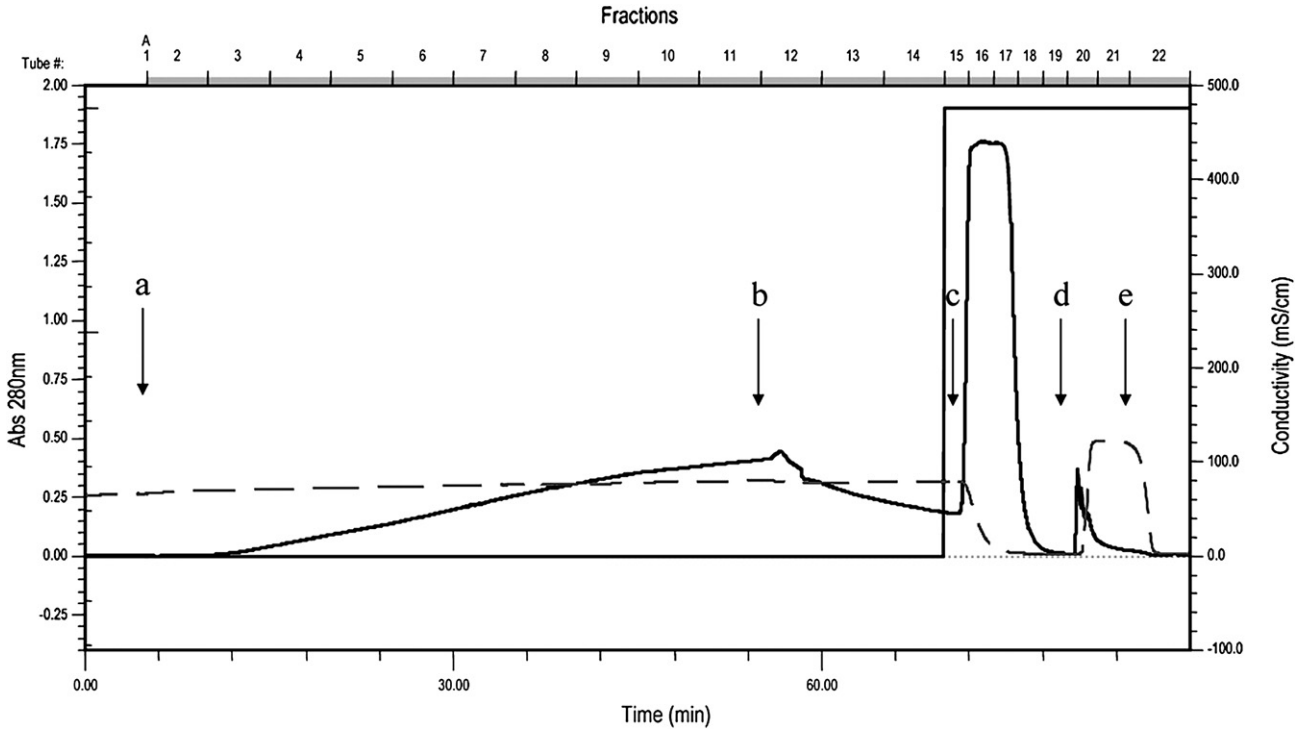



**Fig. 2.** Elution profile for the chromatographic fractionation of a cell culture derived mAb utilizing the Seph–2-QSEA adsorbent. The column was equilibrated with Buffer A (25 mM Tris, 600 mM $Na_2SO_4$, pH 9.0), loaded from the time interval (a) to (b) with a medium sample (50 mL) containing the cell culture derived mAb and then washed from the time interval (b) to (c) solely with Buffer A (15 mL). The bound mAb was eluted by a step change at (c) with Buffer B (10 mL) (25 mM Hepes, pH 7.0). The column was then regenerated by washing from (d) with 0.5 M NaOH (5 mL), and at (e) with Buffer B (5 mL) prior to re-use. A flow rate of 1 mL/min was employed throughout. The monomeric mAb (as assessed by SDS-PAGE analysis) was eluted in fraction no's 16–18 with recovery of 87%, whilst the aggregated mAb (~5% recovery) eluted in fraction no. 20. The solid line is the absorbance at 280 nm and the dashed line is the conductivity ($mScm^{-1}$).

complexity in composition and with scaled up chromatographic systems. These additional results will be presented subsequently once these ongoing investigations are completed.

## 3. Experimental

### 3.1. General

Nuclear magnetic resonance spectra were recorded at 300 MHz ($^1H$) or 75 MHz ($^{13}C$) with a Bruker DPX-300 spectrometer or at 400 MHz ($^1H$) or 100 MHz ($^{13}C$) with a Bruker DRX-400 spectrometer. The NMR spectra refer to solutions in deuterated base-washed ($Na_2CO_3$) $CDCl_3$ where tetramethylsilane (TMS) was used as the internal standard ($\delta$ 0.00 ppm) for $^1H$ spectra and the residual solvent peak used as an internal reference in $^{13}C$ NMR spectra. Chemical shift values ($\delta$) are given in parts per million (ppm) relative to the residual solvent peak (or TMS) and coupling constants are given in hertz (*J* Hz). Infrared spectra were recorded on a Perkin–Elmer 1600 series Fourier Transform infrared spectrometer. Low resolution electrospray ionisation mass spectra (in positive ($ESI^+$) mode) were recorded on a Micromass Platform II API QMS Electrospray mass spectrometer. High-resolution electrospray mass spectra (HRMS) were recorded on a Bruker BioApex 47e Fourier Transform mass spectrometer. Melting points were determined using a Gallenkamp melting point apparatus with a digital thermometer and are uncorrected. Tetrahydrofuran (THF) was distilled from lithium aluminium hydride, stored over sodium wire and distilled from sodium and benzophenone prior to use. Reagents and compounds were purchased from Sigma–Aldrich, Castle Hill, NSW, Australia. Microanalyses were performed by The Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago, Dunedin, New Zealand.

### 3.2. General procedure (A) for preparation of phthalimide derivatives: preparation of 2-[2′-(pyridin-2″-sulfanyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione (3a)

The phthalimides **3a**–**d** were prepared by modifying the procedure outlined by Boschelli et al.[25] as described below for the preparation of phthalimide **3a**.

*N*-(2-Bromoethyl)phthalimide (6.86 g, 27.0 mmol) and $K_2CO_3$ (6.84 g, 49.5 mmol) were added to a solution of 2-mercaptopyridine (2.50 g, 22.5 mmol) in dry acetone (75 mL). The resulting mixture was stirred at reflux for 2 h and then allowed to cool to ambient temperature. The solid was collected by filtration and washed with EtOAc. The filtrate was concentrated in vacuo to give a brown oil. A precipitate formed on standing and the resulting mixture was triturated with 40% EtOAc/hexane to afford the title compound **3a** as a white solid (4.10 g, 64%), mp 94–96 °C (lit.[26] mp 98–99 °C). $\nu_{max}$ (KBr): 3046w, 1770m, 1713s, 1576s, 1455m, 1396s, 1359s, 1237w, 1127m, 1084m, 977w, 862w, 765s, 713s $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 3.52 (t, *J*=6.6 Hz, 2H, H2′); 4.06 (t, *J*=6.6 Hz, 2H, H1′); 6.93 (ddd, *J*=7.3, 4.9, 1.1 Hz, 1H, H5″); 7.17 (apparent dt, *J*=8.1, 1.0 Hz, 1H, H3″); 7.44 (ddd, *J*=8.0, 7.4, 1.9 Hz, 1H, H4′); 7.67–7.85 (m, 4H, H4, H5, H6, H7); 8.36 (ddd, *J*=4.9, 1.8, 0.9 Hz, 1H, H6′). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 28.6 (C2′); 37.9 (C1′); 119.9 (C5″); 122.7 (C3″); 123.6 (C4, C7); 132.5 (C3a, C7a); 134.2 (C5, C6); 136.3 (C4″); 149.8 (C6″); 158.0 (C2″); 168.5 (C1, C3). Mass spectrum ($ESI^+$): *m*/*z* 284.9 $(M+H)^+$ (100%). The $^1H$ NMR spectral data were consistent with literature data.[27]

*3.2.1. 2-[2′-(Pyridin-4″-sulfanyl)ethyl]-1H-isoindole-1,3(2H)-dione (**3b**).* General procedure (A) was followed using *N*-(2-bromoethyl) phthalimide (6.86 g, 27.0 mmol) and 4-mercaptopyridine (2.50 g, 22.5 mmol) in dry acetone (125 mL) to give a brown semi-solid (8.45 g). The slurry was triturated with 30% EtOAc/hexane followed

by acetone/hexane to afford the title compound **3b** as a light brown solid (2.78 g, 43%), mp 143–145 °C (lit.[26] mp 147–148 °C). $\nu_{max}$ (KBr): 3035w, 1767m, 1713s, 1576s, 1456m, 1397s, 1356m, 1188w, 1084m, 974m, 861w, 805m, 717s, 531w $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 3.29 (t, $J$=7.3 Hz, 2H, H2′); 3.99 (t, $J$=7.4 Hz, 2H, H1′); 7.25 (d, $J$=5.9 Hz, 2H, H3″, H5″); 7.72–7.87 (m, 4H, H4, H5, H6, H7); 8.41 (d, $J$=6.0 Hz, 2H, H2″, H6″). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 28.8 (C2′); 37.0 (C1′); 121.2 (C3″, C5″); 123.7 (C4, C7); 132.1 (C3a, C7a); 134.5 (C5, C6); 147.6 (C4″); 149.8 (C2″, C6″); 168.2 (C1, C3). Mass spectrum (ESI$^+$): $m/z$ 285.0 $(M+H)^+$ (100%). The $^1H$ NMR spectral data were consistent with literature data.[28]

*3.2.2. 2-[3″-(Pyridin-2″-ylsulfanyl)propyl]-1H-isoindole-1,3(2H)-dione (**3c**).* General procedure (A) was followed using *N*-(3-bromopropyl) phthalimide (6.63 g, 24.7 mmol), $K_2CO_3$ (6.84 g, 49.5 mmol) and 2-mercaptopyridine (3.03 g, 27.2 mmol) in dry acetone (75 mL) at reflux for 4 h to give a light brown solid (10.5 g). The solid was triturated with hexane and column chromatography of the residual solid ($SiO_2$, hexane/EtOAc, 1:1) gave the title compound **3c** as a light yellow solid (6.94 g, 94%), mp 100–101 °C (lit.[26] mp 103–104 °C). $\nu_{max}$ (KBr): 3451w, 2935w, 1763m, 1707s, 1612w, 1575s, 1456m, 1436m, 1393s, 1348m, 1316m, 1287m, 1244w, 1188w, 1123m, 1103m, 986m, 909w, 883m, 767m, 724s, 618w, 529 m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.11 (m, 2H, H2′); 3.21 (t, $J$=7.3 Hz, 2H, H3′); 3.84 (t, $J$=6.9 Hz, 2H, H1′); 6.92 (ddd, $J$=7.3, 4.9, 1.1 Hz, 1H, H5″); 7.14 (dt, $J$=8.1, 1.0 Hz, 1H, H3″); 7.43 (ddd, $J$=8.1, 7.3, 1.9 Hz, 1H, H4″); 7.71 (m, 2H, H5, H6); 7.83 (m, 2H, H4, H7); 8.30 (ddd, $J$=4.9, 1.9, 1.0 Hz, 1H, H6″). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 27.3 (C3′); 28.9 (C2′); 37.3 (C1′); 119.5 (C5″); 122.5 (C3″); 123.3 (C4, C7); 132.3 (C3a, C7a); 134.0 (C5, C6); 135.9 (C4″); 149.5 (C6″); 158.7 (C2″); 168.5 (C1, C3). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 299.0853. $C_{16}H_{15}N_2O_2S$ requires 299.0854.

*3.2.3. 2-[3′-(Pyridin-4″-ylsulfanyl)propyl]-1H-isoindole-1,3(2H)-dione (**3d**).* General procedure (A) was modified as described by Sohda et al.[26] using *N*-(3-bromopropyl)phthalimide (6.63 g, 24.74 mmol), $K_2CO_3$ (6.84 g, 49.47 mmol) and 4-mercaptopyridine (2.75 g, 24.7 mmol) in DMF (50 mL) at ambient temperature for 3.5 h. The mixture was poured into water (100 mL) and the resulting precipitate collected by vacuum filtration and washed with hexane. Recrystallisation from EtOAc afforded the product **3d** as pale pink crystals (3.57 g, 48%), mp 128–129 °C (lit.[25] mp 128–130 °C). $\nu_{max}$ (KBr): 3466w, 3027w, 2928w, 1780m, 1709s, 1578s, 1535w, 1457m, 1405s, 1377s, 1336m, 1220m, 1171w, 1113w, 1082m, 1007s, 882m, 801m, 715s, 616w, 531m, 494m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.06 (m, 2H, H2′); 3.02 (t, $J$=7.4 Hz, 2H, H3′); 3.86 (t, $J$=6.8 Hz, 2H, H1′); 7.09 (dd, $J$=4.6, 1.6 Hz, 2H, H3″, H5″); 7.74 (m, 2H, H5, H6); 7.85 (m, 2H, H4, H7); 8.38 (dd, $J$=4.6, 1.6 Hz, 2H, H2″, H6″). $^{13}C$ NMR. (75 MHz, $CDCl_3$): $\delta$ 27.9 (C2′); 28.4 (C3′); 37.1 (C1′); 121.0 (C3″, C5″); 123.6 (C4, C7); 132.2 (C3a, C7a); 134.3 (C5, C6); 148.7 (C4″); 149.6 (C2″, C6″); 168.5 (C1, C3). Mass spectrum (ESI$^+$): $m/z$ 299.3 $(M+H)^+$ (100%). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 299.0855. $C_{16}H_{15}N_2O_2S$ requires 299.0854.

### 3.3. General procedure (B) for hydrazinolysis of phthalimide derivatives: preparation of 2-(pyridin-2′-ylsulfanyl)ethanamine (2-PSEA) (6a)

The phthalimides **3a**–**d** were converted to the amines **6a**–**6d** by modifying the procedure outlined by Boschelli et al.[25] as described below for the preparation of 2-PSEA (**6a**).

2-[2′-(Pyridin-2″-sulfanyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione (**3a**) (1.50 g, 5.27 mmol) was suspended in dry EtOH (30 mL) to which hydrazine hydrate (0.33 mL, 6.86 mmol) was added. The suspension was stirred for 16 h at ambient temperature. The mixture was acidified (pH 1) with 2 M HCl and filtered. The filtrate was reduced in vacuo and washed with EtOAc (2×40 mL). The aqueous solution was basified (pH 11) with 2 M NaOH solution and extracted with EtOAc (3×40 mL). The combined organic extract was dried ($MgSO_4$), filtered and evaporated to give a pale yellow solid (0.56 g). Analysis of the crude product by $^1H$ NMR spectroscopy revealed a 4:1 mixture of the desired amine **6a** and a side product. The crude product was re-suspended in dry EtOH (25 mL) and additional hydrazine hydrate was added (0.15 mL). The reaction was stirred at ambient temperature and progress monitored by TLC. After 18 h, additional hydrazine hydrate (0.30 mL) was added and the mixture stirred for a further 8 h. The reaction was worked up as described above to yield the title compound **6a** as a yellow oil (0.48 g, 58%). $\nu_{max}$ (neat): 3363w, 3287w, 3044w, 2925m, 2862w, 1579s, 1556s, 1455s, 1415s, 1283m, 1124s, 1044w, 986m, 854bm, 759s, 724m $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.60 (br s, 2H, $NH_2$); 3.00 (t, $J$=6.2 Hz, 2H, H1); 3.29 (t, $J$=6.4 Hz, 2H, H2); 6.97 (ddd, $J$=7.4, 4.9, 1.1 Hz, 1H, H5′); 7.19 (apparent dt, $J$=8.1, 1.0 Hz, 1H, H3′); 7.46 (ddd, $J$=8.1, 7.4, 1.9 Hz, 1H, H4′); 8.40 (ddd, $J$=4.9, 1.9, 1.0 Hz, 1H, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 34.1 (C2); 41.9 (C1); 119.5 (C5′); 122.6 (C3′); 136.0 (C4′); 149.5 (C6′); 158.7 (C2′). Mass spectrum (ESI$^+$): $m/z$ 154.7 $(M+H)^+$ (100%). The spectral data for **6a** were consistent with literature data.[29]

2-(Pyridin-2′-ylsulfanyl)ethanamine (**6a**) was also prepared following General procedure (A) as described in Section 3.4 using 2-bromopyridine (2.00 g, 12.7 mmol) to give a brown oil (1.54 g). Column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_4OH$, 9:1:0.1) gave the title compound **6a** as a yellow oil (0.95 g, 49%). The spectral data were consistent with those given above.

*3.3.1. 2-(Pyridin-4′-ylsulfanyl)ethanamine (4-PSEA) (**6b**).* General procedure (B) was followed using 2-[2′-(pyridin-4″-sulfanyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione (**3b**) (2.20 g, 7.74 mmol) and hydrazine hydrate (0.49 mL, 10.1 mmol) in dry EtOH (35 mL) for 16 h to give the title compound **6b** as an orange oil (0.99 g, 83%). $\nu_{max}$ (neat): 3358s, 3286s, 3032s, 2926s, 2863m, 1928w, 1731w, 1659m, 1575s, 1538s, 1463s, 1407s, 1321m, 1220s, 1108s, 1066m, 985s, 866s, 804s, 711s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.60 (br s, 2H, $NH_2$); 3.02–3.12 (m, 4H, H1, H2); 7.14 (d, $J$=6.7 Hz, 2H, H3′, H5′); 8.39 (d, $J$=6.3 Hz, 2H, H2′, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 35.0 (C2); 40.8 (C1); 121.2 (C3′, C5′); 148.7 (C4′); 149.5 (C2′, C6′). Mass spectrum (ESI$^+$): $m/z$ 154.7 $(M+H)^+$ (100%). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 155.0640. $C_7H_{11}N_2S$ requires 155.0643. The $^1H$ NMR data were consistent with literature data.[26]

2-(Pyridin-4′-ylsulfanyl)ethanamine (**6b**) was also prepared following General procedure (C) as described in Section 3.4 using 4-chloropyridine hydrochloride (1.94 g, 12.9 mmol) to give a brown oil, which was chromatographed ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_4OH$, 9:1:0.1) to afford the title compound **6b** as a light brown oil (1.73 g, 87%). The spectral data were consistent with those given above.

*3.3.2. 3-(Pyridin-2′-ylsulfanyl)propanamine (2-PSPA) (**6c**).* General procedure (B) was followed using 2-[3′-(pyridin-2″-ylsulfanyl)-propyl]-1*H*-isoindole-1,3(2*H*)-dione (**3c**) (3.00 g, 10.1 mmol) and hydrazine hydrate (1.01 g, 20.1 mmol) in dry EtOH (40 mL) for 17 h to give the title compound **6c** as a yellow oil (1.63 g, 96%). $\nu_{max}$ (neat): 3362m, 3288m, 3044m, 2930s, 2860s, 1661m, 1577s, 1455s, 1415s, 1282m, 1125s, 1089w, 1044w, 986m, 882m, 824m, 758s, 724s, 619m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.53 (br s, 2H, $NH_2$); 1.85 (m, 2H, H2); 2.83 (t, $J$=6.7 Hz, 2H, H1); 3.24 (t, $J$=7.1 Hz, 2H, H3); 6.95 (ddd, $J$=7.3, 4.9, 1.1 Hz, 1H, H5′); 7.16 (dt, $J$=8.1, 1.0 Hz, 1H, H3′); 7.45 (ddd, $J$=8.1, 7.3, 1.9 Hz, 1H, H4′); 8.41 (ddd, $J$=4.9, 1.9, 1.0 Hz, 1H, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 27.3 (C3); 33.4 (C2); 40.9 (C1); 119.3 (C5′); 122.3 (C3′); 135.9 (C4′); 149.4 (C6′); 159.2 (C2′). Mass spectrum (ESI$^+$): $m/z$ 169.0 $(M+H)^+$ (100%). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 169.0793. $C_8H_{13}N_2S$ requires 169.0799. The $^1H$ NMR data were consistent with literature values.[26]

*3.3.3. 3-(Pyridin-4′-ylsulfanyl)propanamine (4-PSPA) (**6d**).* General procedure (B) was followed using 2-[3′-(pyridin-4″-ylsulfanyl)-propyl]-1*H*-isoindole-1,3(2*H*)-dione (**3d**) (2.50 g, 8.38 mmol) and hydrazine hydrate (0.84 g, 16.8 mmol) in dry EtOH (50 mL) for 16 h to give an orange oil (1.63 g). The crude oil was loaded onto a plug of silica, washed ($CH_2Cl_2$/MeOH, 10:1) and the title compound **6d** eluted ($CH_2Cl_2$/MeOH/$NH_3$ in MeOH, 10:1:0.2) as a yellow oil (0.96 g, 68%). $\nu_{max}$ (neat): 3358bs, 3032s, 2933s, 2861s, 1576s, 1538s, 1483s, 1436m, 1409s, 1321m, 1257w, 1221m, 1111m, 1066m, 895m, 804s, 712s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.62 (br s, 2H, $NH_2$); 1.85 (m, 2H, H2); 2.86 (t, *J*=6.8 Hz, 2H, H1); 3.05 (t, *J*=7.2 Hz, 2H, H3); 7.12 (dd, *J*=4.7, 1.6 Hz, 2H, H3′, H5′); 8.37 (dd, *J*=4.7, 1.6 Hz, 2H, H2′, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 28.2 (C3); 32.1 (C2); 41.0 (C1); 120.8 (C3′, C5′); 149.3 (C2′, C6′); 149.3 (C4′). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found *m*/*z* 169.0799. $C_8H_{13}N_2S$ requires 169.0799. The $^1H$ NMR spectral data were consistent with literature values.[26]

### 3.4. General procedure (C) for reaction of 2-amino-ethanethiol·HCl with halogen-substituted *N*-heterocycles

2-Aminoethanethiol hydrochloride (1.1–1.2 equiv) was suspended in dry HMPA (1–4 mL). NaH (dry, 95%) (2.4–2.6 equiv) was added portion-wise over 10–20 min while the reaction mixture was cooled in an ice-cold water bath under a nitrogen atmosphere. The mixture was stirred for 10 min at ambient temperature before portion-wise addition of the desired halogenated heterocycle (1.22–17.5 mmol, 1 equiv) over 10 min. The reaction mixture was stirred at ambient temperature for 2 h–2 days. Water was added slowly to quench the reaction then the aqueous layer was extracted with $CH_2Cl_2$, and the combined organic extract dried ($MgSO_4$), filtered and solvent removed in vacuo to give the crude product. TLC on silica using $CH_2Cl_2$/MeOH/$NH_3$ in MeOH, 9:0.8:0.2 showed the products with $R_f$ values in the range 0.15–0.20.

*3.4.1. Preparation of 2-(pyridin-3′-ylsulfanyl)ethan-amine (**6e**) (3-PSEA).* General procedure (C) was followed employing 3-bromopyridine (1.50 g, 9.49 mmol). Purification of the crude product by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_3$ in MeOH, 9:0.8:0.2) afforded the title compound **6e** as an orange-brown oil (0.73 g, 50%). $\nu_{max}$ (neat): 3362m, 3288m, 3034w, 2923m, 2861w, 1595w, 1568m, 1468s, 1404s, 1322w, 1274w, 1229w, 1189w, 1109m, 1071w, 1019s, 862m, 796s, 706s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.91 (br s, 2H, $NH_2$); 2.92 (m, 2H, H1); 3.03 (m, 2H, H2); 7.22 (ddd, *J*=8.0, 4.8, 0.8 Hz, 1H, H5′); 7.69 (ddd, *J*=8.0, 2.4, 1.6 Hz, 1H, H4′); 8.43 (dd, *J*=4.8, 1.6 Hz, 1H, H6′); 8.60 (dd, *J*=2.4, 0.8 Hz, 1H, H2′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 38.1 (C2); 40.9 (C1); 123.8 (C4′); 133.2 (C3′); 137.7 (C5′); 147.5, 150.8 (C2′, C6′). Mass spectrum (ESI): *m*/*z* 154.9 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found *m*/*z* 155.0642. $C_7H_{11}N_2S$ requires 155.0643. The corresponding 2HCl salt was prepared and obtained as a white solid, mp 217.7 °C (lit.[52] mp 215 °C).

The preparation of **6e** was also carried out by modifying the procedure described by Batmanghelich and Turner.[31]

A suspension of NaH dry, 95% (0.32 g, 13.5 mmol) and 2-amino-ethanethiol hydrochloride (0.80 g, 10.3 mmol) in HMPA (3 mL) was stirred in a cold water bath under a nitrogen atmosphere for 10 min then allowed to warm to ambient temperature. The reaction mixture was cooled again and 3-bromopyridine *N*-oxide (1.50 g, 8.62 mmol) was added. The mixture was stirred at ambient temperature for 3 h. Phosphorus trichloride (1.5 mL) was added and the mixture stirred for 20 h, with $CHCl_3$ (5 mL) added once stirring became inhibited by the thick slurry that formed. The reaction was poured into water (10 mL), basified (pH 9) with 2 M NaOH solution and extracted with $CHCl_3$ (3×20 mL). The combined organic layer was dried ($MgSO_4$), filtered and solvent removed in vacuo and the resulting residue purified as above to give **6e** as an orange-brown oil (0.51 g, 38%).

*3.4.2. 2-(4′-Methylpyridin-2′-ylsulfanyl)ethanamine (**6f**) (4-Me-2-PSEA).* 2-Aminoethanthiol hydrochloride (1.50 g, 13.2 mmol) was added to a suspension of NaH (dry, 95%) (0.79 g, 32.9 mmol) in THF (15 mL). The mixture was stirred for 10 min before the slow addition of HMPA (10 mL). 2-Chloro-4-methylpyridine (1.40 g, 11.0 mmol) was then added slowly and the mixture stirred at ambient temperature for 2 days. The reaction mixture was quenched with water (10 mL) and the THF removed under reduced pressure. The aqueous solution was extracted with $CH_2Cl_2$ (2×10 mL). The combined organic extract was washed with 1 M NaOH (10 mL), dried ($MgSO_4$), filtered and the solvent removed in vacuo to give a brown oil (2.54 g). The crude product was purified by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_4OH$, 9:1:0.1) to yield the title compound **6f** as a light brown oil (0.70 g, 38%). $\nu_{max}$ (neat): 3362m, 3287m, 3048w, 2922s, 2863m, 1594s, 1547s, 1467s, 1372s, 1284m, 1224m, 1122s, 1094s, 1070w, 1015w, 986m, 870s, 816s, 716m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.52 (br s, 2H, $NH_2$); 2.27 (s, 3H, $CH_3$); 2.99 (t, *J*=6.4 Hz, 2H, H1); 3.28 (t, *J*=6.3 Hz, 2H, H2); 6.80 (d, *J*=5.1 Hz, 1H, H5′); 7.03 (s, 1H, H3′); 8.26 (d, *J*=5.1 Hz, 1H, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 20.7 ($CH_3$); 33.0 (C2); 41.4 (C1); 120.9 (C5′); 122.9 (C3′); 147.1 (C4′); 148.9 (C6′); 158.0 (C2′). Mass spectrum ($ESI^+$): *m*/*z* 168.8 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found *m*/*z* 169.0794. $C_8H_{13}N_2S$ requires 169.0799.

*3.4.3. 2-(6′-Methylpyridin-2′-ylsulfanyl)ethanamine (**6g**) (6-Me-2-PSEA).* General procedure (C) was followed employing 2-chloro-6-methylpyridine (1.50 g, 11.76 mmol). The crude brown oil was purified on silica (EtOH) followed by ($CH_2Cl_2$/$NH_3$, 7 M in MeOH, 9:1) to afford the title compound **6g** as a brown oil (1.25 g, 63%). $\nu_{max}$ (neat): 3363m, 3286m, 3056w, 2924s, 2864m, 1566s, 1436s, 1373m, 1256w, 1161s, 1087m, 1035m, 1001m, 975m, 869s, 776s, 729m, 677m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.47 (br s, 2H, $NH_2$); 2.48 (s, 3H, $CH_3$); 2.99 (t, *J*=6.4 Hz, 2H, H1); 3.28 (t, *J*=6.4 Hz, 2H, H2); 6.82 (d, *J*=7.6 Hz, 1H, H3′ or H5′); 7.00 (d, *J*=7.6 Hz, 1H, H3′ or H5′); 7.36 (apparent t, *J*=7.6 Hz, 1H, H4′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 24.6 ($CH_3$); 34.3 (C2); 42.0 (C1); 119.0, 119.4 (C3′, C5′); 136.4 (C4′); 157.6, 158.6 (C2′, C6′). Mass spectrum ($ESI^+$): *m*/*z* 168.9 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found *m*/*z* 169.0794. $C_8H_{13}N_2S$ requires 169.0799.

*3.4.4. 2-(2′-Phenylpyridin-4′-ylsulfanyl)ethanamine (**6h**) (2-Ph-4-PSEA).* General procedure (C) was followed employing 4-chloro-2-phenylpyridine (0.90 g, 4.75 mmol). The crude orange-brown liquid obtained was purified on silica (hexane/EtOAc/EtOH, 3:2:1) followed by ($CH_2Cl_2$/MeOH/$NH_3$ 7 M in MeOH, 85:10:5) to afford the title compound **6h** as a yellow oil (0.90 g, 82%). $\nu_{max}$ (neat): 3372bw, 3037w, 2924w, 1712w, 1663s, 1572s, 1535s, 1498w, 1464m, 1444m, 1381m, 1280w, 1244w, 1221w, 1179w, 1111m, 1074w, 986w, 804m, 774s, 733m, 695s $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.42 (br s, 2H, $NH_2$); 3.05 (m, 2H, H1); 3.14 (m, 2H, H2); 7.09 (dd, *J*=5.3, 1.8 Hz, 1H, H5′); 7.39–7.48 (m, 3H, H3″, H4″, H5″); 7.57 (dd, *J*=1.8, 0.7 Hz, 1H, H3′); 7.94 (m, 2H, H2″, H6″); 8.48 (dd, *J*=5.3, 0.7 Hz, 1H, H6′). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 35.2 (C2); 41.0 (C1); 118.3, 119.5 (C3′, C5′); 127.2, 128.9 (C2″, C3″, C5″, C6″); 129.3 (C4″); 139.3 (C1″); 149.3 (C4′); 149.5 (C6′); 157.6 (C2′). Mass spectrum ($ESI^+$): *m*/*z* 231.1 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found *m*/*z* 231.0961. $C_{13}H_{15}N_2S$ requires 231.0956.

*3.4.5. 2-(6′-Phenylpyridin-2′-ylsulfanyl)ethanamine (**6i**) (6-Ph-2-PSEA).* General procedure (C) was followed employing 2-chloro-6-phenylpyridine (1.80 g, 9.49 mmol). The crude liquid was purified on silica (hexane/$Et_2O$/EtOH, 3:2:1) followed by ($CH_2Cl_2$/MeOH/$NH_3$ 7 M in MeOH, 85:10:5) to afford the title compound **6i** as a yellow oil (1.79 g, 82%). $\nu_{max}$ (neat): 3363bs, 3062s, 2926s, 2868m, 1959w, 1558s, 1496m, 1430s, 1387m, 1312m, 1239w, 1184m, 1159s, 1142s, 1089w, 1063m, 1025m, 982m, 907m, 803s, 758s, 694s $cm^{-1}$.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 1.50 (br s, 2H, $NH_2$); 3.08 (t, $J$=6.3 Hz, 2H, H1); 3.41 (t, $J$=6.3 Hz, 2H, H2); 7.13 (dd, $J$=7.8, 0.8 Hz, 1H, H3′); 7.40–7.48(m, 4H, H5′, H3″, H4″, H5″); 7.53 (apparent t, $J$=7.8 Hz, 1H, H4′); 8.01 (m, 2H, H2″, H6″). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 34.2 (C2); 42.0 (C1); 116.0, 121.0 (C3′, C5′); 126.9, 128.9 (C2″, C3″, C5″, C6″); 129.2 (C4″); 136.8 (C4′); 139.2 (C1″); 156.9, 158.3 (C2′, C6′). Mass spectrum (ESI$^+$): $m/z$ 231.3 $(M+H)^+$ (100%). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 231.0953. $C_{13}H_{15}N_2S$ requires 231.0956.

*3.4.6. 2-(6′-Methoxypyridin-2′-ylsulfanyl)ethanamine (**6j**) (6-OMe-2-PSEA).* General procedure (C) was followed employing 2-chloro-6-methoxypyridine (1.50 g, 10.5 mmol). The crude brown liquid obtained was dissolved in dry ether (3 mL) and dry MeOH (3 mL) and a saturated solution of HCl in ether (3 mL) was added. The resulting precipitate was collected by filtration, washed with dry ether and obtained as a beige solid (1.05 g). The solid was dissolved in water, basified (pH 8–9) with a saturated $K_2CO_3$ solution and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to give the title compound **6j** as a brown oil (0.58 g, 30%). $\nu_{max}$ (neat): 3372m, 2946m, 2862m, 1568s, 1462s, 1405s, 1347w, 1288s, 1260s, 1190m, 1147s, 1074m, 1025s, 984m, 884s, 785s, 729m cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.45 (br s, 2H, $NH_2$); 3.02 (t, $J$=6.3 Hz, 2H, H1); 3.27 (t, $J$=6.3 Hz, 2H, H2); 3.93 (s, 3H, $OCH_3$); 6.42 (dd, $J$=8.1, 0.7 Hz, 1H, H3′ or H5′); 6.79 (dd, $J$=7.5, 0.7 Hz, 1H, H3′ or H5′); 7.38 (dd, $J$=8.1, 7.5 Hz, 1H, H4′). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 34.2 (C2); 41.8 (C1); 53.3 ($OCH_3$); 105.8 (C5′); 114.6 (C3′); 138.7 (C4′); 155.7 (C2′); 163.7 (C6′). Mass spectrum (ESI$^+$): $m/z$ 185.0 $(M+H)^+$ (100%). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 185.0741. $C_8H_{13}N_2OS$ requires 185.0749.

A similar reaction was undertaken in DMF at 60 °C for 23 h and gave **6j** in 42% yield.

### 3.5. Preparation of 2-(4′-nitropyridin-2′-ylsulfanyl)-ethanamine (6k) (4-NO$_2$-2-PSEA)

*tert*-Butyl (2-sulfanylethyl)carbamate (1.38 g, 7.81 mmol) was dissolved in dry THF (20 mL) under a nitrogen atmosphere. NaH (dry, 95%) (0.24 g, 10.2 mmol) was added portion-wise over 45 min and the mixture was then cooled to −78 °C. A solution of 2-chloro-4-nitropyridine *N*-oxide (1.50 g, 8.59 mmol) in dry THF (40 mL) was added dropwise over 45 min to the cold carbamate solution. The mixture was warmed to ambient temperature over 2 h, stirred for a further 2 h and the THF was removed under reduced pressure. The residue was treated with $CHCl_3$ (50 mL) followed by dropwise addition of $PCl_3$ (3 mL). The reaction mixture was stirred at ambient temperature for 16 h and then poured slowly into water (50 mL). The organic layer was separated and the aqueous layer washed with $CHCl_3$ (4×15 mL). The aqueous layer was basified (pH 8.5–10) with 2 M NaOH solution and extracted several times with $CH_2Cl_2$. The combined organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to give the title compound **6k** as a red-brown oil (ca. 95% pure) (1.06 g, ca. 68%). Preparative TLC ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_3$ in MeOH, 9:0.8:0.2) using a sample of crude oil gave the pure title compound **6k** as a yellow oil. $\nu_{max}$ (neat): 3372m, 3083m, 2927m, 2870m, 1600m, 1560s, 1531s, 1453m, 1427w, 1355s, 1234m, 1153s, 1100m, 1073w, 1015w, 982w, 910m, 881m, 838m, 761s, 734s, 679m cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.52 (br s, 2H, $NH_2$); 3.03 (t, $J$=6.4 Hz, 2H, H1); 3.35 (t, $J$=6.4 Hz, 2H, H2); 7.66 (dd, $J$=5.4, 2.0 Hz, 1H, H5′); 7.89 (dd, $J$=2.0, 0.7 Hz, 1H, H3′); 8.66 (dd, $J$=5.4, 0.7 Hz, 1H, H6′). $^{13}$C NMR (50 MHz, $CDCl_3$): $\delta$ 34.5 (C2); 41.6 (C1); 111.8 (C5′); 115.1 (C3′); 151.5 (C6′); 153.9, 162.9 (C2′, C4′). Mass spectrum (ESI$^+$): $m/z$ 200.0 $(M+H)^+$ (30%), 183.0 (100). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 200.0494. $C_7H_{10}N_3O_2S$ requires 200.0494.

*3.5.1. 2-(2′-Chloropyridin-4′-yl-sulfanyl)ethanamine (2-Cl-4-PSEA).* General procedure (C) was followed employing 2-chloro-4-nitropyridine (0.20 g, 1.26 mmol). The crude yellow liquid obtained was dissolved in dry ether (3 mL) and dry MeOH (3 mL) to which a saturated solution of HCl in ether (2 mL) was added. The resulting precipitate was filtered, washed with ice-cold dry ether and collected as a yellow solid (132 mg). The yellow solid was dissolved in water (5 mL), basified (pH 8–9) with saturated $K_2CO_3$ solution and extracted with $CH_2Cl_2$ (3×5 mL). The combined organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to yield 2-Cl-4-PSEA as a brown oil (85 mg, 36%). $\nu_{max}$ (neat): 3362bs, 2927s, 2868m, 2457w, 1569s, 1523s, 1456s, 1428m, 1371s, 1284m, 1230m, 1151s, 1111m, 1082s, 986s, 795s, 750m cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 1.38 (br s, 2H, $NH_2$); 3.02–3.11 (m, 4H, H1, H2); 7.05 (dd, $J$=5.4, 1.7 Hz, 1H, H5′); 7.15 (dd, $J$=1.7, 0.4 Hz, 1H, H3′); 8.15 (dd, $J$=5.4, 0.4 Hz, 1H, H6′). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 35.0 (C2); 40.7 (C1); 119.8, 120.5 (C3′, C5′); 149.0 (C6′); 151.9, 152.3 (C2′, C4′). Mass spectrum (ESI$^+$): $m/z$ 190.8 (M ($^{37}$Cl)+H)$^+$ (36%), 188.8 (M ($^{35}$Cl)+H)$^+$ (100). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 189.0245. $C_7H_{10}ClN_2S$ requires 189.0253.

### 3.6. 2-(Quinolin-4′-ylsulfanyl)ethanamine (10a) (4-QSEA)

General procedure (C) was followed using 4-chloroquin-oline (0.20 g, 1.22 mmol). The crude brown oil obtained was dissolved in dry ether (3 mL) and dry MeOH (3 mL) and treated with a saturated solution of HCl in ether (3 mL). The amine salt precipitated and was collected by filtration and washed with dry ether to give **10a**·2HCl as a white solid (240 mg, 71%), mp 221–224 °C, (lit.[52] mp 222–226 °C). The solid was then dissolved in water, basified with $K_2CO_3$ and extracted with $CH_2Cl_2$ (3×10 mL). The combined organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to give the title compound **10a** as a brown oil (130 mg, 52%). $\nu_{max}$ (neat): 1563m, 1376w, 933m, 822w, 810w, 667w, 643m, 630m cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.97 (br s, 2H, $NH_2$); 3.12 (m, 2H, H1); 3.24 (m, 2H, H2); 7.24 (d, $J$=4.8 Hz, 1H, H3′); 7.56 (apparent ddd, $J$=8.4, 6.9, 1.4 Hz, 1H, H6′ or H7′); 7.72 (apparent ddd, $J$=8.4, 6.9, 1.4 Hz, 1H, H6′ or H7′); 8.07 (ddd, $J$=8.4, 1.4, 0.6 Hz, 1H, H5′ or H8′); 8.17 (ddd, $J$=8.4, 1.4, 0.6 Hz, 1H, H5′ or H8′); 8.72 (d, $J$=4.8 Hz, 1H, H2′). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 35.4 (C2); 40.7 (C1); 116.5 (C3′); 123.8, 126.5, 129.9, 130.2 (C5′, C6′, C7′, C8′); 127.0 (C4a'); 147.1, 147.7 (C4′, C8a'); 149.4 (C2′). Mass spectrum (ESI$^+$): $m/z$ 205.0 $(M+H)^+$ (15%). HRMS (ESI$^+$, MeOH): $(M+H)^+$, found $m/z$ 205.0794. $C_{11}H_{13}N_2S$ requires 205.0799.

Reaction of 4-chloroquinoline (1.50 g, 9.17 mmol) with sodium ethoxide (34.8 mmol) and 2-aminoethanethiol hydrochloride (1.56 g, 13.8 mmol) in dry ethanol (40 mL) at reflux for 21 h gave a 60:40 mixture of **10a** and 4-ethoxyquinoline. Column chromatography on silica ($CH_2Cl_2$/MeOH/$NH_4OH$, 9:2:0.2) gave 4-ethoxyquinoline[53] as a light brown oil (0.47 g, 30%). Trituration of a more polar fraction gave *N*-[2-(quinolin-4-yl-sulfanyl)ethyl]quinolin-4-amine as a white solid (0.32 g, 11%), mp 217–219 °C. $^1$H NMR. (300 MHz, $CDCl_3$): $\delta$ 3.51 (t, $J$=6.3 Hz, 2H, $SCH_2$); 3.77 (apparent q, $J$=6.3 Hz, 2H, $CH_2NH$); 5.43 (br s, 1H, NH); 6.46 (d, $J$=5.3 Hz, 1H, H3); 7.26 (d, $J$=4.8 Hz, 1H, H3″); 7.41 (apparent ddd, $J$=8.3, 7.0, 1.3 Hz, 1H, ArH); 7.58 (apparent ddd, $J$=8.3, 7.0, 1.3 Hz, 1H, ArH); 7.64 (apparent ddd, $J$=8.4, 7.0, 1.4 Hz, 1H, ArH); 7.67 (m, 1H, ArH); 7.74 (apparent ddd, $J$=8.4, 7.0, 1.4 Hz, 1H, ArH); 8.00 (m, 1H, ArH); 8.10 (ddd, $J$=8.4, 1.3, 0.6 Hz, 1H, ArH); 8.18 (ddd, $J$=8.4, 1.4, 0.6 Hz, 1H, ArH); 8.58 (d, $J$=5.3 Hz, 1H, H2) 8.69 (d, $J$=4.8 Hz, 1H H2″). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 30.8 ($SCH_2$); 41.8 ($CH_2NH$); 99.2, 117.3 (ArCH); 119.0 (ArC); 119.4, 123.9, 125.2, 127.0 (ArCH); 127.1 (ArC); 129.5, 130.3, 130.4 (ArCH); 145.8, 148.0, 148.7, 149.1 (ArC); 149.4, 151.1 (ArCH). Mass spectrum (ESI): $m/z$ 332.2 $(M+H)^+$ (100%). HRMS (ESI$^+$, $CH_2Cl_2$:MeOH, 1:4): $(M+H)^+$, found $m/z$ 332.1217. $C_{20}H_{18}N_3S$ requires 332.1221.

*3.6.1. 2-(Quinolin-2′-ylsulfanyl)ethanamine (**10b**) (2-QSEA).* General procedure (C) was followed employing 2-chloroquinoline (2.00 g,

12.2 mmol). The crude brown oil obtained was dissolved in dry ether (5 mL) and treated with a saturated solution of HCl in ether (3 mL). The resulting precipitate was collected by filtration and washed with ice-cold dry ether to give the 2HCl salt of **10b** as a yellow solid (2.82 g), mp 210–211 °C. Anal. Calcd for $C_{11}H_{12}N_2S\cdot 2HCl$: C, 47.66; H, 5.09; N, 10.105. Found: C, 47.35; H, 5.19; N, 9.98%. The solid was dissolved in water, basified (pH 8–9) with a saturated $K_2CO_3$ solution and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to give the title compound **10b** as a brown oil (2.10 g, 84%). $\nu_{max}$ (neat): 3358m, 3281m, 3055m, 2925m, 2862m, 1614s, 1593s, 1555s, 1497s, 1452m, 1420s, 1376w, 1294s, 1138s, 1089s, 1017w, 942m, 861m, 817s, 780m, 750s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.67 (br s, 2H, $NH_2$); 3.08 (bt, $J$=6.2 Hz, 2H, H1); 3.45 (t, $J$=6.2 Hz, 2H, H2); 7.20 (d, $J$=8.6 Hz, 1H, H3′); 7.40 (apparent ddd, $J$=8.1, 6.9, 1.2 Hz, 1H, H6′ or H7′); 7.61 (apparent ddd, $J$=8.4, 7.0, 1.5 Hz, 1H, H6′ or H7′); 7.68 (apparent dd, $J$=8.1, 1.5 Hz, 1H, H5′ or H8′); 7.84 (dd, $J$=8.7, 0.6 Hz, 1H, H4′); 7.91 (apparent dd, $J$=8.4, 1.2 Hz, 1H, H5′ or H8′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 33.8 (C2); 41.9 (C1); 121.2 (C3′); 125.4 (ArCH); 126.1 (C4a'); 127.7, 128.1, 129.7 (ArCH); 135.5 (C4′); 148.4 (C8a'); 158.9 (C2′). Mass spectrum ($ESI^+$): $m/z$ 205.1 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 205.0802. $C_{11}H_{13}N_2S$ requires 205.0799.

*3.6.2. 2-(Isoquinolin-1′-ylsulfanyl)ethanamine (**10c**) (1-IQSEA).* General procedure (C) was followed using 1-chloroisoquinoline (2.00 g, 12.2 mmol). The crude yellow liquid obtained was dissolved in dry ether (6 mL) and dry MeOH (3 mL). A saturated solution of HCl in ether (3 mL) was added, the resulting precipitate collected by filtration, washed with ice-cold dry ether and dried in vacuo to yield the HCl salt of the title compound **10c** as a white solid (3.12 g, 92%), mp dec >120 °C. Anal. Calcd for $C_{11}H_{12}N_2S\cdot 2HCl\cdot 1.2H_2O$: C, 44.2; H, 5.5; N, 9.4. Found: C, 44.2; H, 5.7; N, 9.3%. The white solid (1.50 g) was dissolved in water, basified (pH 8–9) with saturated $K_2CO_3$ solution and extracted with $CH_2Cl_2$ (3×15 mL). The combined organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to yield the title compound **10c** as a yellow oil (1.09 g, 99%). $\nu_{max}$ (neat): 3366m, 3293m, 3051m, 2926m, 2870m, 2806w, 1766w, 1619s, 1589s, 1551s, 1494s, 1450m, 1402m, 1373m, 1337s, 1301s, 1261s, 1226m, 1201m, 1184m, 1150s, 1066m, 1025m, 989s, 909m, 866m, 816s, 745s, 675s, 648s $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.43 (br s, 2H, $NH_2$); 3.05 (t, $J$=6.4 Hz, 2H, H1); 3.47 (t, $J$=6.4 Hz, 2H, H2); 7.29 (dd, $J$=5.7, 0.6 Hz, 1H, H4′); 7.51 (apparent ddd, $J$=8.4, 6.9, 1.4 Hz, 1H, H6′ or H7′); 7.61 (apparent ddd, $J$=8.2, 6.9, 1.2 Hz, 1H, H6′ or H7′); 7.70 (br d, $J$=8.2 Hz, 1H, H5′ or H8′); 8.20 (br d, $J$=8.4 Hz, 1H, H5′ or H8′); 8.27 (d, $J$=5.7 Hz, 1H, H3′). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.7 (C2); 41.8 (C1); 117.3 (C4′); 124.6, 127.0, 127.1 (ArCH); 127.2 (C8a'); 130.3 (ArCH); 135.5 (C4a'); 141.8 (C3′); 159.0 (C1′). Mass spectrum ($ESI^+$): $m/z$ 205.2 $(M+H)^+$ (100%), 188.2 (100). HRMS ($ESI^+$, MeOH): $(M^+)$, found $m/z$ 204.0714. $C_{11}H_{12}N_2S$ requires 204.0721.

*3.6.3. 2-(Pyrimidin-2′-ylsulfanyl)ethanamine (**10d**) (2PymSEA).* General procedure (C) was followed employing 2-chloropyrimidine (2.00 g, 17.5 mmol). The crude brown oil was purified by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_4OH$, 9:1:0.1) and the title compound **10d** was obtained as a yellow oil (1.90 g, 70%). $\nu_{max}$ (neat): 3362m, 3289m, 3030w, 2930m, 2866w, 1587s, 1564s, 1548s, 1459w, 1427m, 1380s, 1257w, 1202s, 1072w, 1016w, 870m, 803m, 774s, 748s, 630s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.65 (br s, 2H, $NH_2$); 3.03 (t, $J$=6.4 Hz, 2H, H1); 3.26 (t, $J$=6.4 Hz, 2H, H2); 6.96 (t, $J$=4.8 Hz, 1H, H5′); 8.50 (d, $J$=4.8 Hz, 2H, H4′, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 35.0 (C2); 41.7 (C1); 116.7 (C5′); 157.4 (C4′, C6′); 172.4 (C2′). Mass spectrum ($ESI^+$): $m/z$ 156.2 $(M+H)^+$ (75%), 139.2 (100). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 156.0593. $C_6H_{10}N_3S$ requires 156.0595. The $^1H$ NMR spectral data were consistent with literature values.[26]

*3.6.4. 2-(Pyrazin-2′-ylsulfanyl)ethanamine (**10e**) (2-PyzSEA).* General procedure (C) was followed employing 2-chloropyrimidine (2.00 g, 17.5 mmol). A portion of the crude material (4.41 g) was purified on silica, (hexane:$Et_2O$:EtOH, 3:2:1) followed by ($CH_2Cl_2$/MeOH/$NH_3$ 7 M in MeOH, 85:10:5) to give the title compound **10e** as a yellow oil (1.77 g, 85%). $\nu_{max}$ (neat): 3358m, 3288m, 3058m, 2929m, 2865m, 1597m, 1562m, 1506s, 1460s, 1385s, 1285m, 1237w, 1181w, 1129s, 1072w, 1048s, 1006s, 835s, 760m $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.54 (br s, 2H, $NH_2$); 3.02 (t, $J$=6.4 Hz, 2H, H1); 3.30 (t, $J$=6.4 Hz, 2H, H2); 8.21 (d, $J$=2.7 Hz, 1H, H6′); 8.34 (dd, $J$=2.7, 1.6 Hz, 1H, H5′); 8.47 (d, $J$=1.6 Hz, 1H, H3′). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.6 (C2); 41.6 (C1); 139.6 (C5′); 143.9, 144.3 (C3′, C6′); 156.8 (C2′). Mass spectrum ($ESI^+$): $m/z$ 156.1 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 156.0590. $C_6H_{10}N_3S$ requires 156.0595. The corresponding 2HCl salt was prepared and obtained as a white solid, mp 162–164 °C (lit.[31] mp 161 °C).

*3.6.5. 2-(2′,2″:6′,2‴-Terpyridin-4′-ylsulfanyl)-ethanamine (**10f**) (4-TerPSEA).* 2-Aminoethanethiol hydrochloride (0.76 g, 6.7 mmol) was dissolved in dry DMF (6 mL) and NaH (dry, 95%) (0.38 g, 15.7 mmol) was added portion-wise over 20 min while the reaction mixture was cooled in an ice-cold water bath under a nitrogen atmosphere. The mixture was stirred for 10 min at ambient temperature before portion-wise addition of 4′-chloro-2,2′:6′,2″-terpyridine[39] (1.50 g, 8.6 mmol) over 10 min. The reaction mixture was heated at 50 °C for 3 h. Water was added slowly to quench the reaction then the aqueous layer was extracted with $CH_2Cl_2$ (4×20 mL) and the combined washed with saturated NaCl solution (6×10 ml). The organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to give the product as a light beige solid (1.64 g, 95%), mp 98–99 °C (lit.[38] mp 93.5–94.5 °C). $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 3.10 (t, $J$=6.4 Hz, 2H, H1); 3.29 (t, $J$=6.4 Hz, 2H, H2); 7.33 (ddd, $J$=7.5, 4.8, 1.2 Hz, 2H, H5″, H5‴); 7.85 (apparent td, $J$=7.5, 1.8 Hz, 2H, H4″, H4‴); 8.37 (s, 2H, H3′, H5′); 8.60 (dt, $J$=8.0, 1.1 Hz, 2H, H3″, H3‴); 8.68 (ddd, $J$=8.0, 4.8, 1.0 Hz, 2H, H6″, H6‴). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 35.3 (C2); 41.1 (C1); 118.3 (ArCH); 121.6 (ArCH); 124.1 (ArCH); 137.1 (ArCH); 149.3 (ArCH); 150.9 (ArC); 155.35 (ArC); 156.1 (ArC). Mass spectrum ($ESI^+$): $m/z$ 309.1 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found: $m/z$ 309.1167. $C_{17}H_{17}N_4S$ requires 309.1174. The NMR data were consistent with literature data.[38]

### 3.7. Preparation of 3-(pyridin-2′-yl)propanethiol (13a) (2-MPP)

2-Picoline (3.62 g, 38.9 mmol) was dissolved in dry THF (100 mL) containing TMEDA (0.5 mL). The solution was cooled to −78 °C under a nitrogen atmosphere before dropwise addition of *n*-butyllithium, 0.88 M solution in hexanes, (66 mL, 58 mmol). The solution was then allowed to warm to −10 °C before being re-cooled to −78 °C. Thiirane (3.5 mL, 58 mmol) was added dropwise to the reaction mixture. The solution was stirred for 30 min at −78 °C and then allowed to warm to ambient temperature. After stirring overnight the reaction mixture was quenched with saturated $NH_4Cl$ (100 mL). THF was removed under reduced pressure and the aqueous residue extracted with $CH_2Cl_2$ (3×40 mL). The combined organic extract was dried ($Na_2SO_4$), filtered and solvent removed under reduced pressure to give a brown oil (8.7 g). The crude product was purified by distillation using a Kugelrohr apparatus to afford the title compound **13a** as a yellow oil (2.68 g, 45%), bp 150 °C (oven)/0.5 mmHg. $\nu_{max}$ (neat): 3376bm, 3065m, 3008m, 2929s, 2857s, 2533w, 1592s, 1567s, 1475s, 1434s, 1348w, 1289m, 1150m, 1095w, 1050m, 994m, 751s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.40 (t, $J$=7.9 Hz, 1H, SH); 2.06 (m, 2H, H2); 2.57 (apparent q, $J$=7.4 Hz, 2H, H1); 2.91 (t, $J$=7.4 Hz, 2H, H3); 7.10 (ddd, $J$=7.5, 4.9, 1.2 Hz, 1H, H5′); 7.15 (d, $J$=7.8 Hz, 1H, H3′); 7.59 (apparent td, $J$=7.7, 1.8 Hz, 1H, H4′); 8.52 (ddd, $J$=4.9, 1.8, 0.9 Hz, 1H, H6′). $^{13}C$

NMR (75 MHz, $CDCl_3$): $\delta$ 24.3 (C1); 33.9, 36.9 (C2, C3); 121.3, 123.0 (C3′, C5′); 136.5 (C4′); 149.5 (C6′); 161.2 (C2′). Mass spectrum ($ESI^+$): $m/z$ 305.4 $(disulfide+H)^+$ (50%), 153.9 $(M+H)^+$ (100). HRMS ($ESI^+$, MeOH): $(M+Na)^+$, found $m/z$ 176.0510. $C_8H_{11}NNaS$ requires 176.0510.

*3.7.1. 3-(Pyridin-4′-yl)propanethiol (**13b**) (4-MPP).* 4-Picoline (3.29 g, 35.3 mmol) was reacted with *n*-butyllithium, 0.88 M solution in hexanes (60 mL, 53 mmol) and TMEDA (0.5 mL) in dry THF (100 mL) as described for **13a** (2-MPP) above. Dropwise addition at −78 °C of thiirane (3.2 mL, 53 mmol) to the reaction mixture resulted in a colour change from red-orange to bright yellow. The solution was stirred for 30 min at −78 °C, allowed to warm to ambient temperature and stirred overnight. Workup as described for **13a** (2-MPP) gave a brown oil (8.08 g). The crude oil was partially purified by distillation using a Kugelrohr apparatus. The fractions contained both the side product 2-butyl-4-methylpyridine[53] as a yellow oil (0.13 g, 2%) and crude 4-MPP, which was further purified by column chromatography ($SiO_2$, EtOAc) to give **13b** as a yellow oil (0.56 g, 10%), bp 150 °C (oven)/0.5 mmHg (lit.[44] 84–85 °C/0.25 mmHg). $\nu_{max}$ (neat): 3385bm, 3026m, 2932s, 1670m, 1602s, 1558m, 1496m, 1416s, 1295w, 1255w, 1220m, 1070w, 993m, 791m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.38 (t, $J$=7.9 Hz, 1H, SH); 1.95 (m, 2H, H2); 2.54 (apparent q, $J$=7.3 Hz, 2H, H1); 2.74 (t, $J$=7.6 Hz, 2H, H3); 7.11 (d, $J$=6.0 Hz, 2H, H3′, H5′); 8.49 (d, $J$=6.0 Hz, 2H, H2′, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 24.0 (C1); 33.7, 34.4 (C2, C3); 124.0 (C3′, C5′); 150.0 (C2′, C6′); 150.3 (C4′). Mass spectrum ($ESI^+$): $m/z$ 305.3 $(disulfide+H)^+$ (100%), 153.9 $(M+H)^+$ (73).

4,4′-(Disulfanediyldipropane-3,1-diyl)dipyridine was also obtained as an orange oil (0.48 g, 4%). $\nu_{max}$ (neat): 3388bm, 3026m, 2931s, 2859m, 1602s, 1558m, 1496w, 1447m, 1415s, 1219m, 1069w, 993m, 792m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.03 (m, 4H, $CH_2$); 2.66 (t, $J$=7.1 Hz, 4H, $CH_2$); 2.72 (t, $J$=7.6 Hz, 4H, $CH_2$); 7.11 (d, $J$=6.0 Hz, 4H, ArCH); 8.50 (d, $J$=6.0 Hz, 4H, ArCH). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 29.5, 33.7, 37.8 ($CH_2$); 124.0 (ArCH); 149.9 (ArCH); 150.4 (ArC). Mass spectrum ($ESI^+$): $m/z$ 305.3 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 305.1140. $C_{16}H_{21}N_2S_2$ requires 305.1146.

*3.7.2. 3-(Pyridin-3′-yl)propanethiol (**13c**) (3-MPP).* 3-Picoline (3.19 g, 34.3 mmol) was dissolved in dry THF (100 mL) containing TMEDA (0.5 mL). Lithium diisopropylamide (LDA) was prepared by dissolving diisopropylamine (6.2 mL, 44.5 mmol) in dry THF (20 mL) under a nitrogen atmosphere, cooling to 0 °C and adding *n*-butyllithium, 0.88 M solution in hexanes (50 mL, 44.5 mmol) dropwise. The LDA solution was stirred for a further 40 min at 0 °C and added dropwise to the picoline solution at −78 °C under nitrogen. After complete addition (30 min) the solution was stirred for a further 10 min at −78 °C. The mixture was allowed to warm to 0 °C before re-cooling to −78 °C. Thiirane (3.0 mL, 51 mmol) was added dropwise to the reaction mixture. The solution was stirred for a further 15 min at −78 °C and then allowed to warm to ambient temperature and stirred overnight. Workup as described for **13a** gave a brown oil (9.30 g). The crude product was partially purified by distillation using a Kugelrohr apparatus. Column chromatography ($SiO_2$, EtOAc) afforded the title compound **13c** as a yellow oil (0.15 g, 3%), bp 150 °C (oven)/0.5 mmHg (lit.[40] 92–99 °C/0.15 mmHg). $\nu_{max}$ (neat): 3384bw, 2929s, 2859m, 1664w, 1575m, 1478m, 1423s, 1299w, 1190w, 1103w, 1028m, 796m, 715s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.38 (t, $J$=7.8 Hz, 1H, SH); 1.94 (m, 2H, H2); 2.55 (m, 2H, H1); 2.75 (t, $J$=7.6 Hz, 2H, H3); 7.22 (m, 1H, H5′); 7.51 (m, 1H, H4′); 8.44–8.46 (m, 2H, H2′, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 23.9 (C1); 31.5, 35.1 (C2, C3); 123.5 (C5′); 136.1 (C4′); 136.7 (C3′); 147.6, 150.0 (C2′, C6′). Mass spectrum ($ESI^+$): $m/z$ 305.1 $(disulfide+H)^+$ (100%), 153.6 $(M+H)^+$ (49). The $^1H$ NMR spectral data of **13c** were consistent with literature data.[54]

### 3.8. General procedure (D) for the preparation of PMSEA derivatives

2-Aminoethanethiol hydrochloride (12.2–24.4 mmol) and the 2-, 3- or 4-(chloromethyl)pyridine hydrochloride (6.1–12.2 mmol) were added to a solution of NaOH (24.4–48.8 mmol) in EtOH (20–40 mL) with ice bath cooling. The reaction mixture was stirred for 30 min before the ice bath was removed and the mixture then stirred at ambient temperature for 2.5 h. The EtOH was removed under reduced pressure and water (25 mL) was added to the resulting residue. The aqueous solution was extracted with $CH_2Cl_2$ (3×25 mL) and the combined organic layer was washed with brine (10 mL), dried ($K_2CO_3$), filtered and solvent removed in vacuo to afford a crude oil.

*3.8.1. 2-(Pyridin-2′-ylmethylsulfanyl)ethanamine (**15a**) (2-PMSEA).* Reaction of 2-(chloromethyl)pyridine hydrochloride (1.00 g, 6.10 mmol), following the general procedure (D) gave a crude oil (1.18 g). Column chromatography ($SiO_2$, $CH_2Cl_2$:MeOH:$NH_4OH$, 9:2:0.2) of the crude oil gave the title compound **15a** as a yellow oil (0.92 g, 89%). $\nu_{max}$ (neat): 3356bs, 2922s, 1592s, 1568s, 1472s, 1435s, 1308w, 1214w, 1153w, 1088w, 1049m, 995m, 891m, 792m, 751s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.75 (br s, 2H, $NH_2$); 2.61 (t, $J$=6.3 Hz, 2H, H2); 2.85 (t, $J$=6.3 Hz, 2H, H1); 3.84 (s, 2H, Ar$CH_2$S); 7.17 (ddd, $J$=7.5, 4.9, 1.2 Hz, 1H, H5′); 7.38 (apparent dt, $J$=7.8, 0.9 Hz, 1H, H3′); 7.66 (apparent td, $J$=7.7, 1.8, Hz, 1H, H4′); 8.52 (ddd, $J$=4.9, 1.8, 0.9 Hz, 1H, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 34.7, 36.8, 39.9 (C1, C2, Ar–CH2–S); 121.0 (C5′); 122.1 (C3′); 135.8 (C4′); 148.2 (C6′); 157.9 (C2′). Mass spectrum ($ESI^+$): $m/z$ 168.8 $(M+H)^+$ (100%). The $^1H$ NMR data of **15a** were consistent with literature data.[55]

*3.8.2. 2-(Pyridin-3′-ylmethylsulfanyl)ethanamine (**15b**) (3-PMSEA).* Reaction of 3-(chloromethyl)pyridine hydrochloride (1.00 g, 6.10 mmol) following general procedure (D) gave a crude orange oil (1.08 g). Column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_4OH$, 9:2:0.2) of the crude orange oil afforded the title compound **15b** as a yellow oil (0.77 g, 75%). $\nu_{max}$ (neat): 3356bs, 2918s, 1591s, 1576s, 1478s, 1423s, 1253w, 1192m, 1100m, 1027s, 874m, 713s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.64 (br s, 2H, $NH_2$); 2.54 (t, $J$=6.3 Hz, 2H, H2); 2.85 (bt, $J$~5.9 Hz, 2H, H1); 3.70 (s, 2H, Ar$CH_2$S); 7.26 (ddd, $J$=7.9, 4.8, 0.8 Hz, 1H, H5′); 7.69 (m, 1H, H4′); 8.50 (apparent dd, $J$=4.8, 1.6 Hz, 1H, H6′); 8.53 (br d, $J$=2.3 Hz, 1H, H2′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 33.3, 35.6, 40.9 (C1, C2, Ar$CH_2$S); 123.7 (C5′); 134.3 (C3′); 136.5 (C4′); 148.7, 150.1 (C2′, C6′). Mass spectrum ($ESI^+$): $m/z$ 168.8 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 169.0795, $C_8H_{13}N_2S$ requires 169.0799.

*3.8.3. 2-(Pyridin-4′-ylmethylsulfanyl)ethanamine (**15c**) (4-PMSEA).* Reaction of 4-(chloromethyl)pyridine hydrochloride (2.00 g, 12.20 mmol) following general procedure (D) gave a crude brown oil (2.12 g). Column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH/$NH_4OH$, 9:2:0.2) of the crude brown oil afforded the title compound **15c** as a yellow oil (1.93 g, 94%). $\nu_{max}$ (neat): 3356bs, 2919s, 1601s, 1560m, 1495w, 1415s, 1219w, 1068m, 994m, 882m, 819m $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.75 (br s, 2H, $NH_2$); 2.53 (t, $J$=6.3 Hz, 2H, H2); 2.84 (t, $J$=6.3 Hz, 2H, H1); 3.67 (s, 2H, Ar$CH_2$S); 7.26 (d, $J$=6.0 Hz, 2H, H3′, H5′); 8.54 (d, $J$=6.0 Hz, 2H, H2′, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 35.1, 35.8, 40.9 (C1, C2, Ar$CH_2$S); 124.0 (C3′, C5′); 147.7 (C4′); 150.1 (C2′, C6′). Mass spectrum ($ESI^+$): $m/z$ 168.7 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 169.0796. $C_8H_{13}N_2S$ requires 169.0799.

### 3.9. Preparation of 2-(2′-pyridin-2″-ylethylsulfanyl)-ethanamine (17a) (2-PESEA)

2-(2′-Pyridin-2″-ylethylsulfanyl)ethanamine (**17a**) was synthesised according to the method described by Kaasjager et al.[44]

2-Aminoethanethiol hydrochloride (2.16 g, 19.0 mmol) was added to a solution of 2-vinylpyridine (2.00 g, 19.0 mmol) in dry EtOH (50 mL). The mixture was stirred at reflux for 2 h before addition of 1 M NaOH in EtOH (20 mL) and reflux continued for a further 30 min. The cloudy solution was filtered to remove NaCl and the filtrate was concentrated in vacuo to give a brown oil (3.66 g). The residue was dissolved in water (20 mL) and the aqueous solution was extracted with $CH_2Cl_2$ (3×20 mL). The combined organic extract was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the title compound **17a** as a brown oil (3.30 g, 95%). $\nu_{max}$ (neat): 3361s, 3286m, 3064m, 3008m, 2918s, 2856s, 1592s, 1568s, 1474s, 1436s, 1271m, 1211m, 1151m, 1051m, 995m, 846bm, 754s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.41 (br s, 2H, $NH_2$); 2.62 (t, $J$=6.5 Hz, 2H, H2); 2.87 (t, $J$=6.4 Hz, 2H, H1); 2.94 (m, 2H, H1′); 3.07 (m, 2H, H2′); 7.13 (ddd, $J$=7.5, 4.9, 1.2 Hz, 1H, H5″); 7.18 (apparent dt, $J$=7.8, 1.0 Hz, 1H, H3″); 7.60 (apparent td, $J$=7.7, 1.9 Hz, 1H, H4″); 8.54 (ddd, $J$=4.9, 1.8, 0.9 Hz, 1H, H6″). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 31.5 (C1′); 36.7 (C2); 38.7 (C2′); 41.3 (C1); 121.6 (C5″); 123.3 (C3″); 136.5 (C4″); 149.5 (C6″); 160.1 (C2″). Mass spectrum (ESI): $m/z$ 182.8 $(M+H)^+$ (100%). The spectral data were consistent with literature data.[44]

*3.9.1. 2-(2′-Pyridin-4″-ylethylsulfanyl)ethanamine (**17b**) (4-PESEA).* 2-Aminoethanethiol hydrochloride (2.16 g, 19.0 mmol) was reacted with 4-vinylpyridine (2.00 g, 19.0 mmol) in dry EtOH (50 mL) at reflux for 2 h as described above for **17a** to give a crude yellow oil (3.18 g). The crude product was purified to remove remaining 4-vinylpyridine using a plug of silica (EtOAc/hexane, 3:1, containing 10% EtOH). Elution (EtOAc/hexane, 3:1, containing 10% $NH_3$ in MeOH) to gave the desired compound **17b** as a yellow oil (2.86 g, 82%). $\nu_{max}$ (neat): 3359bs, 3070m, 2920s, 2858m, 1602s, 1559m, 1416s, 1321w, 1275w, 1222m, 1070w, 994m, 805s $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 1.47 (br s, 2H, $NH_2$); 2.60–2.94 (m, 8H, H1, H2, H1′, H2′); 7.13 (d, $J$=6.0 Hz, 2H, H3″, H5″); 8.52 (d, $J$=6.0 Hz, 2H, H2″, H6″). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.0 (C1′); 35.5, 36.4 (C2, C2′); 41.3 (C1); 123.8 (C3″, C5″); 149.1 (C4″); 149.7 (C2″, C6″). Mass spectrum ($ESI^+$): $m/z$ 182.9 $(M+H)^+$ (100%). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 183.0944. $C_9H_{15}N_2S$ requires 183.0956.

### 3.10. Preparation of 2-(pyridin-2′-ylsulfanyl)ethanol (19) (2-PSEOH)

2-Mercaptoethanol (1.09 g, 13.9 mmol) was dissolved in dry HMPA (3 mL) to which NaH (0.40 g, 16.7 mmol) was added portionwise for 30 min under a nitrogen atmosphere. After complete addition, the mixture was stirred for 10 min before 2-bromopyridine (2.00 g, 12.7 mmol) was added drop-wise. The mixture was stirred at ambient temperature for 18 h and then at 40 °C for 24 h. Water (20 mL) was added slowly and the aqueous solution extracted with $CH_2Cl_2$ (3×15 mL). The combined organic extract was dried ($MgSO_4$), filtered and solvent removed in vacuo to give a yellow oil (5.53 g). Column chromatography ($SiO_2$, ether/hexane, 4:1) gave the title compound (**19**) as a yellow oil (ca. 95% pure) (1.54 g, ca. 72%). $\nu_{max}$ (neat): 3362bs, 2929s, 2864s, 1580s, 1557s, 1455s, 1415s, 1283s, 1232m, 1149s, 1045s, 1016s, 942m, 878w, 758s, 724s $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 3.31 (t,=$J$ 5.5 Hz, 2H, H2); 3.93 (t, $J$=5.5 Hz, 2H, H1); 7.00 (ddd, $J$=7.4, 5.0, 1.1 Hz, 1H, H5′); 7.25 (dt, $J$=8.1, 1.0 Hz, 1H, H3′); 7.48 (ddd, $J$=8.0, 7.4, 1.9 Hz, 1H, H4′); 8.35 (ddd, $J$=5.0, 1.8, 1.0 Hz, 1H, H6′). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 33.9 (C2); 63.2 (C1); 119.9, 122.8 (C3′, C5′); 136.4 (C4′); 148.9 (C6′); 158.8 (C2′). Mass spectrum (ESI): $m/z$ 155.8 $(M+H)^+$ (55%), 137.8 (100). HRMS ($ESI^+$, MeOH): $(M+H)^+$, found $m/z$ 156.0534. $C_7H_{10}NOS$ requires $m/z$ 156.0483. The spectral data were consistent with literature data.[46]

A similar reaction at ambient temperature for 25 h gave a 54% conversion ($^1H$ NMR spectroscopy) to the alcohol **19**.

### 3.11. Ligand immobilisation

Epichlorohydrin activated Sepharose 6 Fast Flow® was prepared by the method described by Jiang et al.[3] Sepharose 6 Fast Flow® (500 g) (Amersham Pharmacia Biotechnology, Uppsala, Sweden; now GE Healthcare) was filtered and washed extensively with distilled water (5×volume of wet gel, 2.5 L). The gel was suction dried and transferred to a Schott bottle. A 2 M NaOH solution (500 mL) containing $NaBH_4$ (0.937 g) (1.875 mg/mL) was added and the resulting suspension mixed vigorously for 2 h on an Axyos orbital shaker. Epichlorohydrin (300 mL) was added and the gel slurry was gently agitated for a further 6 days. The epoxy-activated resin was collected by vacuum filtration and washed with distilled water (2.5 L). The activated resin was stored in a 20% (v/v) aqueous ethanol at 4 °C until required for ligand immobilisation.

The ligands were attached to the activated Sepharose based on the methods described by Jiang et al.[3] A 0.2 M solution of the ligand was prepared by dissolving the heterocyclic compound in an appropriate solvent (e.g., 25% or 50% (v/v) aqueous methanol). The suction dried epichlorohydrin-activated resin was added to the solution and the suspension mixed thoroughly on a rotating wheel for 5 days at ambient temperature. The resulting adsorbent was collected by vacuum filtration, washed with the appropriate solvent (ca. 10 bed volumes) followed by distilled water (ca. 10 bed volumes). The adsorbent was stored in 20% (v/v) aqueous ethanol solution at 4 °C.

The extent of ligand immobilisation onto the epichlorohydrin-activated Sepharose 6 Fast Flow® was determined by the nitrogen elemental analysis (Dairy Technical Services Ltd., Melbourne, Australia). In brief, the adsorbent (approximately 10 g wet weight) was collected by filtration, washed with 25% (v/v) aqueous acetone followed by 50% (v/v), then 75% (v/v), and finally 100% acetone (ca. two bed volumes) and dried in vacuo to a constant weight. The dried resin was accurately weighed and then analysed for total nitrogen content to give the amount of immobilised ligand per gram of dry resin. A typical standard deviation for replicates of these analyses was ±14 μmol/g dry gel.

### 3.12. Static and dynamic adsorption studies

The static adsorption studies were carried out by incubating the adsorbents with different concentrations of the purified monoclonal antibody (mAb) under a previously defined buffer condition (pH 9.0, 600 mM $Na_2SO_4$). Each incubation (550 μL total volume) contained Tris buffer (55 μL), mAb solution (110 μL), adsorbent slurry (20 μL), 1 M $Na_2SO_4$ (330 μL) and $H_2O$ (35 μL). The mAb solutions were prepared at the following concentrations: 330, 550, 770, 935, 1155, 1320, 1540 μg/mL in 25 mM sodium acetate pH 5, 220 mM sodium chloride buffer.

Following incubation, the free mAb concentrations in the supernatants were measured in triplicate by the BCA protein assay. The values of the maximum binding capacity (as mg mAb/mL adsorbent) were determined from the derived isotherm using established procedures. The BCA protein assays were carried out according to the manufacturer's specifications with the propriety reagent A and reagent B (Pierce, Rockford, USA). An aliquot of the supernatant from each incubation sample (20 μL) was placed in triplicate in the wells of a 96-well microplate. A mixture of reagent A (50 parts) and reagent B (1 part) was then prepared and 200 μL of the resulting solution added to each well. The plate was incubated at 37 °C for 30 min to allow the colour to develop. The absorbance was then read on a Bio-Rad Microplate Reader Model 3550 at 595 nm and the protein concentration in the supernatant sample determined from a standard curve using bovine serum albumin as the control protein. The recoveries were determined from the ratio of mass balances for the loaded and eluted mAb fractions, whilst

the $Q_{max}$ values and the corresponding $R^2$ values for the static and dynamic studies were determined from the derived fit of the experimental data to a Langmuir isotherm based on methods described previously.[56,57]

The chromatographic dynamic adsorption studies were carried out using the adsorbents (1.0 mL) packed into glass Econo-Columns, connected to a Bio-Rad BioLogic Duo Flow Chromatographic System The column was equilibrated with Buffer A (25 mM Tris, 600 mM $Na_2SO_4$, pH 9.0) (5 mL) at a flow rate of 1 mL/min. A crude media sample containing the cell culture derived mAb was diluted fourfold with Buffer A. The sample was then loaded onto the column at a flow rate of 1 mL/min and the column washed with Buffer A (15 mL). The bound monomeric mAb was eluted with Buffer B (10 mL) (25 mM Hepes pH 7.0). The column was regenerated by elution with 0.5 M NaOH (5 mL), and Buffer B (5 mL). A flow rate of 1 mL/min was employed throughout.

The chromatographic fractions were monitored at 280 nm using UV–vis spectrometry. The mAb concentration in the eluted fractions and the total recovery were determined using a standard curve prepared by recording the absorbance of a sample of the pure mAb at concentrations of 0.25, 0.50, 0.75, 1.0, 1.5 mg/mL. The protein purity confirmed by SDS-PAGE analysis.

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## Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.11.003.

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