

# CASE STUDY: SECONDARY AMINE SYNTHESIS USING BOUND REAGENTS, SOLID PHASE EXTRACTION (SPE) AND FLASH CHROMATOGRAPHY

## INTRODUCTION

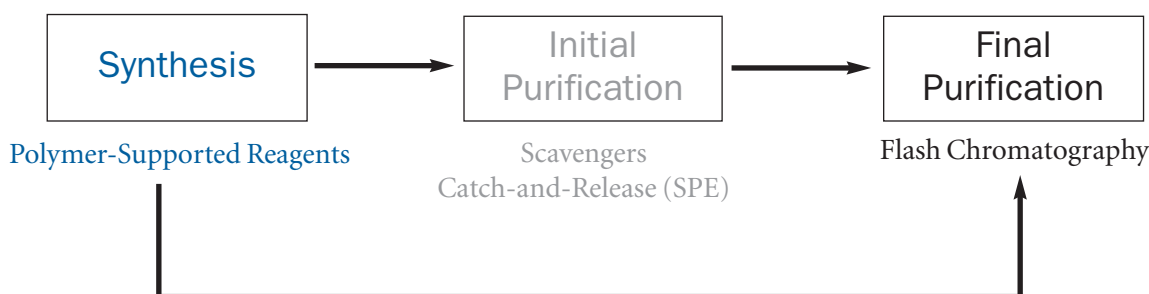
Synthesis and purification of organic compounds is a fundamental component in pharmaceutical research and development. Medicinal chemists are confronted with the growing demand for high purity compounds with ever-increasing structural complexity. Producing a wide variety of organic compounds in high purity and under tight timelines remains the major challenge. Any compound synthesized needs to be purified from all other ingredients such as excess reagents, catalysts, byproducts and solvents. In many cases, it is the isolation and purification steps that account for the major time and cost of an organic synthesis. Thus, an efficient synthetic strategy must address the efficacy of both the synthesis and purification steps. However, there have been few reports addressing both synthesis and purification in an integrated approach.

It is within this context we sought to evaluate an integrated synthesis and purification approach using polymer-supported reagents and scavengers, SPE and flash chromatography. In this case study we demonstrated the proof of the concept for an integrated strategy by successful synthesis and isolation of the secondary amine, N-butylphenethylamine using bound reagents, SPE and flash chromatography.

## INTEGRATED SYNTHESIS AND PURIFICATION STRATEGY

The workflow strategy for integrated organic synthesis and purification is outlined in **Scheme 1**. The strategy involves the sequential use of polymer-supported reagents and scavengers, “catch-and-release” purification and automated flash chromatography for final purification. This integrated approach to synthesis and purification is modular by nature and tunable to variable chemistry requirements. Depending on the chemistry type, a synthesis and purification workflow may skip the initial purification step or may not require the final purification. Chromatographic purification is often needed after an organic reaction is performed. This may require preliminary purification, traditionally performed by liquid-liquid extraction or solvent exchange. An important component of this workflow strategy is to develop flow through methods to integrate pre-chromatography workup with the final purification.

**Scheme 1: Synthesis and Purification Workflow**



### **Synthesis using Polymer-supported Reagents<sup>1</sup>**

Polymer-supported reagents are functional polymers designed to perform synthetic transformations in the same way as their corresponding unbound counterparts. The bound reagents can be used in excess to drive a reaction to completion and are particularly useful in multi-step reactions. Both the spent and excess reagent remain immobilized and therefore can be easily removed by filtration.

### **Initial Purification using Polymer-supported Scavengers and SPE<sup>1</sup>**

Polymer bound scavengers are functional polymers designed to purify a crude reaction mixture by selectively reacting with excess reagents and reaction byproducts. They are added after the reaction is complete. Combinations of bound scavengers containing mutually incompatible functional groups such as an acid and a base may be used in a one-pot purification process. Catch-and-release purification or scavenging using a flow through column format provides rapid and highly efficient method of purification.

### **Final Purification using Automated Flash Chromatography**

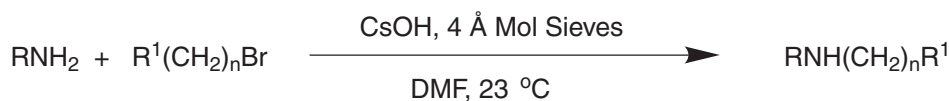
Flash chromatography remains a widely used technique for separation of organic compounds, particularly those containing compounds having similar structural motifs and polarity profiles. One important example in this category is a mixture containing primary, secondary and tertiary amines. Thus, depending on the chemistry type, a reaction may require final purification by flash chromatography using Argonaut's FlashMaster™ II Flash Chromatography System.<sup>2</sup>

## **SYNTHESIS OF SECONDARY AMINES**

Synthesis of amines remains paramount in view of their versatile biological and medicinal applications. Amines and their carboxamide derivatives are the most prevalent structural moieties found in the comprehensive medicinal chemistry database.<sup>3</sup> Among the various concepts for the synthesis of secondary amines, selective mono-N-alkylation of primary amines remains by far the most direct approach. However, the conventional base-promoted alkylation reactions of primary amines using alkyl halides routinely suffer from poor chemoselectivity due to the competing over-alkylation<sup>4</sup> producing a mixture of amines that are difficult to purify.

A chemoselective mono-N-alkylation of primary amines in the presence of cesium hydroxide and 4 Å molecular sieves has been reported recently in the literature (**Scheme 2**).<sup>5</sup> The reactions were performed in DMF using various alkyl bromides to produce secondary amines along with variable amounts of tertiary amines. Nevertheless, the chemoselectivity of the reaction was found to be very sensitive to the presence of moisture and the handling of a strong hygroscopic compound such as cesium hydroxide was problematic. The workup and isolation process required aqueous quenching, liquid-liquid extraction and chromatographic separation.

**Scheme 2: Chemoselective N-Alkylation of Primary Amines**



In our case study for integrated organic synthesis and purification, we have chosen the synthesis of the secondary amine, N-butylphenethylamine as the probe reaction. The N-alkylation reactions on the primary amine,  $\beta$ -phenethylamine were performed using both cesium hydroxide and a polymer-supported tetraalkylammonium hydroxide base.<sup>6</sup> We sought to probe this reaction as it would produce a mixture of primary, secondary and tertiary amines, and provide an opportunity to explore all three steps of the workflow strategy described earlier (**Scheme 1**).

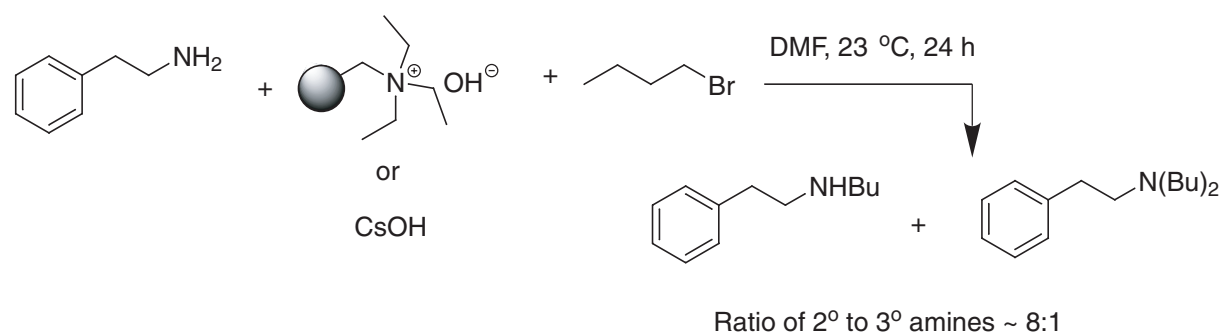
## RESULTS AND DISCUSSION

### Synthesis and Purification Steps

The integrated synthesis and purification of the secondary amine, N-butylphenethylamine involved the following steps:

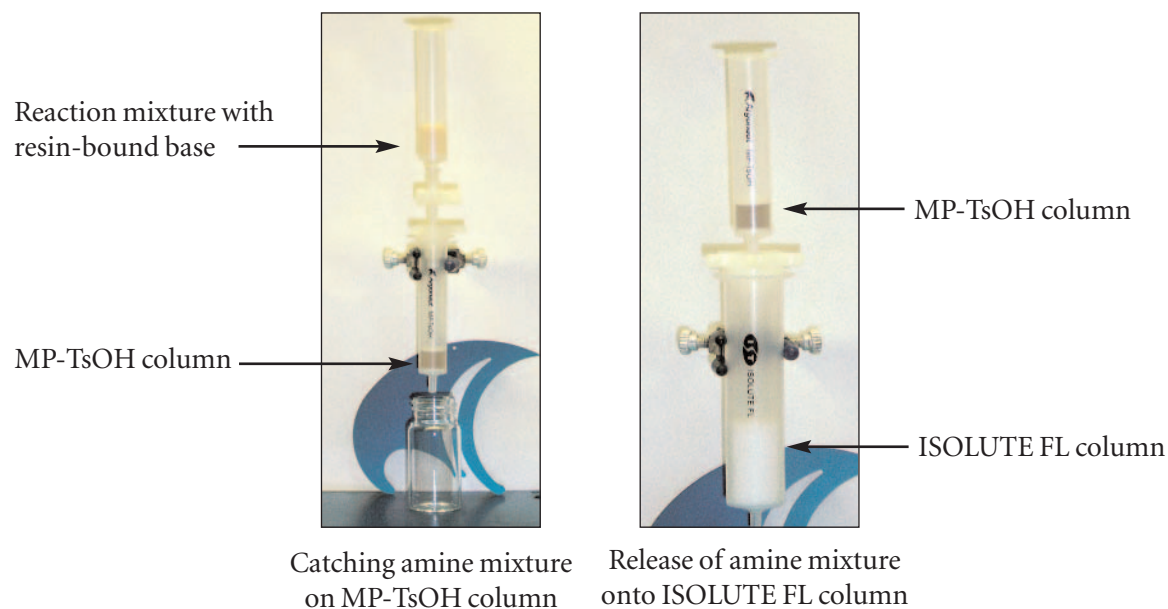
1. N-alkylation reactions on  $\beta$ -phenethylamine were performed with n-butyl bromide using both cesium hydroxide and a polymer-supported tetraalkylammonium hydroxide base<sup>6</sup> (**Scheme 3**).
2. The product mixture was isolated by simple filtration.
3. Initial purification of the amine mixture was performed by catch-and-release purification using a MP-TsOH column<sup>7</sup> that removes non-basic impurities and allows solvent (DMF) exchange.
4. The amine mixture was loaded onto an ISOLUTE<sup>®</sup> FL column<sup>8</sup> and the solvent was removed.
5. Finally, the primary, secondary and tertiary amines were separated by flash chromatography using Argonaut's FlashMaster II system<sup>2</sup>, and an ISOLUTE Flash SI column.<sup>9</sup>

**Scheme 3: Chemoselective Mono-N-Alkylation of Phenethylamine**



The cesium hydroxide promoted N-alkylation of  $\beta$ -phenethylamine with n-butylamine was performed following the protocol reported in literature.<sup>5</sup> The reaction proceeded at room temperature in dry DMF in the presence of 4 Å molecular sieves. Analysis of the crude reaction mixture by GC confirmed a mixture of primary, secondary and tertiary amines in the ratio of 2:6:2. However, the chemoselectivity of secondary amine formation was found to be very sensitive to the presence of water. The reaction was also carried out using a polymer-supported tetraalkylammonium hydroxide base<sup>6</sup> and the crude product mixture had a similar composition. The polymer-supported base was easier to handle than the moisture-sensitive cesium hydroxide and was utilized for further studies. In both cases, the reactions may be performed in filtration columns for the convenience of filtration with agitation by rotary wheel devices or orbital shakers.

**Figure 1: Amine Purification and Solvent Removal**



The main objective of this study was to develop flow through protocols to integrate reaction workup with the final chromatographic purification step. The initial post-reaction workup involved filtration of the reaction mixture onto a MP-TsOH column.<sup>7</sup> MP-TsOH columns are designed specifically for flow through purification of amines. In general, 1.5–2.0 equiv of MP-TsOH were used with respect to the basic components present in the reaction mixture. This SPE process retained the mixture of primary, secondary and tertiary amines on the MP-TsOH column, with THF washes used to remove the non-basic reactants and byproducts. The method also effected convenient removal of the high boiling DMF solvent from the product mixture. The MP-TsOH column was then attached to an ISOLUTE FL column<sup>8</sup> and the amine mixture released onto the florisil column using a 4 M solution of ammonia in methanol. Typically, the total volume of methanol-ammonia solution required for quantitative release of the amine mixture was found to be around 3 mL for a 0.5 mmol reaction scale. An ISOLUTE FL 5 g/25 mL column was found appropriate to absorb this amount of methanol. The florisil column was then dried using a vacuum dessicator. The flow through catch-and-release processes are shown in **Figure 1**.

The final purification step involved chromatographic separation of the amine mixture using Argonaut's FlashMaster II system.<sup>2</sup> The dried ISOLUTE FL column containing the amine mixture was connected in series with an ISOLUTE Flash SI 5 g/25 mL column<sup>9</sup> (**Figure 2**). Absorbing the amine mixture onto the florisil that imitates the dry loading process prior to flash chromatography, facilitates amine transfer to the flash column. The silica column was pre-equilibrated on-line with ethyl acetate. Flash chromatography was then performed on-line using a gradient of ethyl acetate and ethanol containing 2% (v/v) ammonia. The less polar tertiary amine was eluted first followed by the secondary amine product, while the unreacted primary amine remained on the column. The analytical purity of the isolated amine products was confirmed by GC analysis (**Figure 3**).

Figure 2: Flash Chromatography on FlashMaster II System

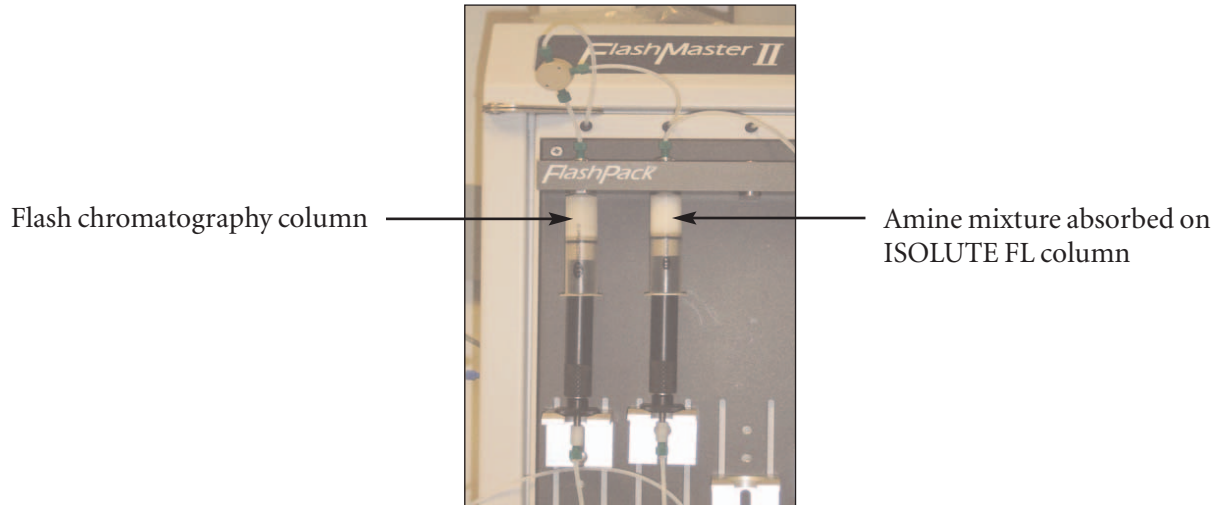
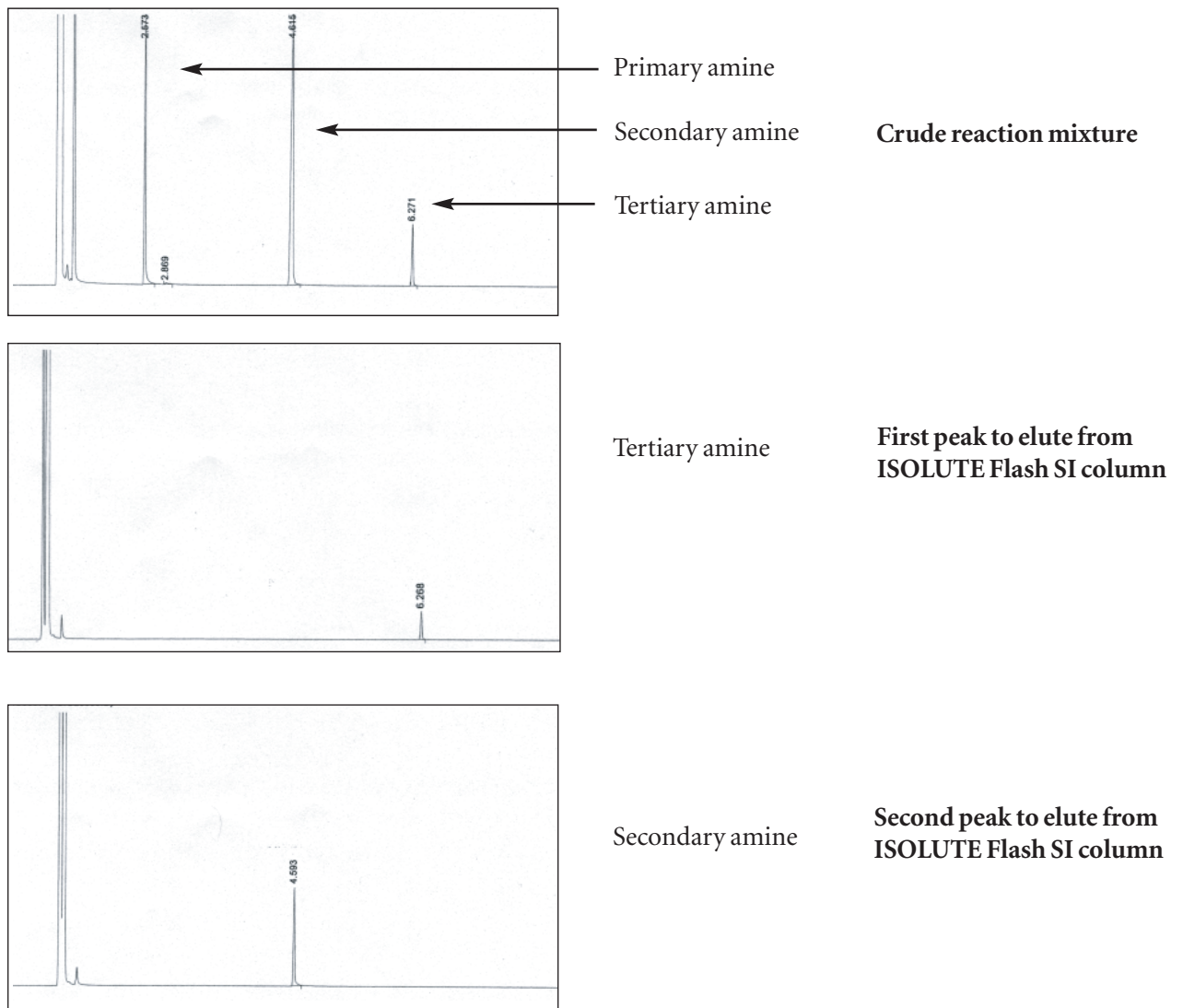


Figure 3: Purification Results: GC analysis



## SUMMARY

In summary, we have demonstrated an integrated synthesis, workup and purification approach for secondary amines. Particularly noteworthy is the development of flow through methods that integrate workup and purification processes. The synthesis involved chemoselective mono-N-alkylation of the primary amine,  $\beta$ -phenethylamine using a polymer-supported tetraalkylammonium hydroxide resin. The initial purification utilized SPE of the amine mixture on a MP-TsOH column that exchanged the high boiling DMF solvent with more volatile methanol and removed any excess alkyl halide. Release of the amine mixture onto an ISOLUTE FL column facilitated amine loading onto the flash column. Finally, the separation of the amine mixture was accomplished using Argonaut's FlashMaster II system.

## EXPERIMENTAL

### ***N-Alkylation of Phenethylamine using Polymer-supported Base:***<sup>6</sup>

To a mixture of the polymer-supported base (0.4 g, 0.72 mmol, 1.8 mmol/g) and DMF (2.5 mL) in an ISOLUTE SG empty reservoir, 6 mL<sup>10</sup> was added a 1.0 M DMF solution of  $\beta$ -phenethylamine (0.5 mL, 0.5 mmol) and a 1.0 M DMF solution of n-butyl bromide (0.6 mL, 0.6 mmol). The reaction mixture was agitated at room temperature for 16 hours using a rotary wheel device. The reaction mixture was then filtered onto a MP-TsOH column (500 mg/6 mL, 3.1 mmol/g) and washed with THF (2 x 3 mL). The MP-TsOH column was next attached onto an ISOLUTE FL 5 g/25 mL column. The amine mixture was released from the MP-TsOH column and absorbed on the florisil column using a 4 M solution of ammonia in methanol (2 mL) and the MP-TsOH column was washed with methanol (2 x 0.5 mL). The florisil column was detached, dried and connected to an ISOLUTE Flash SI 5 g/25 mL column on FlashMaster II system. Flash chromatography using a gradient of ethyl acetate and ethanol containing 2% (v/v) ammonia eluted the tertiary amine, N, N-dibutylphenethylamine followed by the secondary amine, N-butylphenethylamine. The isolated and purified amines were characterized by comparison of their GC with authentic samples.

### ***N-Alkylation of Phenethylamine using Cesium Hydroxide:***

To a suspension of activated molecular sieves (4 Å, 0.15 g) and cesium hydroxide monohydrate (0.085 g, 0.5 mmol) in DMF (2 mL) in an ISOLUTE SG empty reservoir, 6 mL<sup>10</sup> was added a 1.0 M DMF solution of  $\beta$ -phenethylamine (0.5 mL, 0.5 mmol). After the mixture was stirred for 30 minutes at room temperature, a 1.0 M DMF solution of n-butyl bromide (0.6 mL, 0.6 mmol) was added and the reaction was further agitated for 16 hours. The reaction mixture was then processed as described above.

## REFERENCES

1. See for example: Bhattacharyya, S.; Rana, S.; Gooding, O. W.; Labadie, J. *Tetrahedron Lett.* **2003**, *44*, 4957 and references 1-2 cited therein; Synthesis and Purification Catalog, Argonaut Technologies, 2003.
2. Synthesis and Purification Catalog, Argonaut Technologies, 2003, p. 111, Part Number: 11-000-F2SPC.
3. Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Comb. Chem.* **1999**, *1*, 55.
4. March, J. *Advanced Organic Chemistry*, Wiley, New York, **1992**, p. 411; Salvatore, R. N.; Yoon, C. H.; Jung, K. *W Tetrahedron*, **2001**, *57*, 7785.
5. Salvatore, R. N.; Nagle, A. S.; Jung, K. W. *J. Org. Chem.* **2002**, *67*, 674.
6. The polymer-supported tetraalkylammonium hydroxide resin was prepared from the corresponding chloride resin by anion exchange using an aqueous solution of NaOH. The dried resin had a typical loading of 1.6–1.8 mmol/g.
7. Synthesis and Purification Catalog, Argonaut Technologies, 2003, pp 93–96, Part Number: 800477-0050-C.
8. Synthesis and Purification Catalog, Argonaut Technologies, 2003, p. 132, Part Number: 712-0500-E.
9. Synthesis and Purification Catalog, Argonaut Technologies, 2003, pp. 116–117, Part Number: 450-0500-E.
10. Synthesis and Purification Catalog, Argonaut Technologies, 2003, pp. 136–137, Part Number: 120-1103-C.

© 2004 Argonaut Technologies, Inc. All rights reserved. ISOLUTE is a registered trademark and FlashMaster is a trademark of Argonaut Technologies, Inc.

116-702

### Japanese Headquarters

Itsuwa Bldg. 6F,  
2-26-9, Nishigotanda,  
Shinagawa-ku, Tokyo, 141-0031, Japan  
T: +81-3-5719-1239  
F: +81-3-5719-1203

### European Headquarters

New Road, Hengoed, Mid Glamorgan  
United Kingdom, CF82 8AU  
T: +44 (0) 1443 811811  
F: +44 (0) 1443 816552

