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A Practical Guide for Buffer-Assisted Isolation and Purification of Primary, Secondary, and Tertiary Amine Derivatives from Their Mixture

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Abstract:

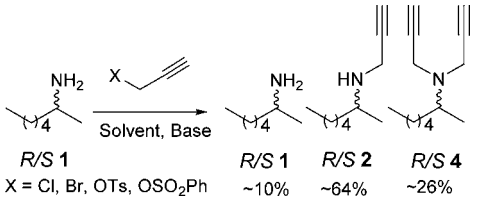
A number of reactions involving primary amines such as N-alkylation, reductive amination, Mannich reaction, etc. produce mixtures of primary, secondary, and tertiary amines. The isolation and purification of individual constituents of these mixtures is often carried out by column chromatography or fractional distillation. For an industrial large-scale process, it would be desirable to avoid column chromatography and distillation (if the product is temperature sensitive) without significantly losing the product yield. We have devised an elegant method which uses relatively inexpensive buffer medium of varying pH to selectively separate primary, secondary, and tertiary amines.

Introduction

There are a number of reactions that involve formation of compounds with alkyl/aryl substitution on N atom where side products are often formed. Examples include N-alkylation,[1] reductive amination,[2] Mannich reactions[3] involving primary amines or ammonia. Purification of the desired product may not be challenging, but it indeed is tedious and time-consuming when it involves column chromatography and/or fractional distillation. These methods of purification are not particularly desirable of large scale industrial processes.[4] Thus, there exists a need for a simple method of purification of mixtures containing amines with varying degree of substitution on N atom.

We are immensely interested in (*R*)-*N*-(prop-2-ynyl)-heptan-2-amine (**2**) and (*R*)-*N*-methyl-*N*-(prop-2-ynyl)heptan-2-amine (**3**) which possess cellular rescue properties.[5,6] We have demonstrated that these compounds are preclinical candidates for treatment and prevention of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, stroke, amyotrophic lateral sclerosis, etc.[5,6] Compound **2**, which is also a precursor for compound **3**,[5] is usually prepared from the reactions of a propargyl halide (bromide, chloride) or a sulfonate (benzenesulfonate, tosylate) with (*R*)-2-heptanamine (**1**) (Scheme 1).[5] In these reactions, the desired product is invariably contaminated by dipropargyl-substituted compound (**4**) and the unreacted starting material **1** and the alkylating agent.[5] For small-scale laboratory synthesis, the purification can be performed by column chromatography. Column chromatography is an almost impossible option for large-scale preparation of compounds in industrial plants.[4] We were investigating a process that can efficiently be used for the purification of the desired amine from the crude reaction mixture containing starting materials and byproduct(s). It should be noted that the removal of nonbasic constituents of the crude reaction products can easily be performed by acid extraction.[7] We herein describe an atypical, extremely efficient and simple procedure of separation of individual constituents from a mixture of amines with varying degrees of substitution on N with a high degree of purity using a mixture of **1**, **2**, and **4** as our model example (Figure 1).

Scheme 1. **Synthetic route followed to prepare *racemic* 2**



Results and Discussion

Our procedure for separation of a mixture of primary, secondary, and tertiary amines employs the difference in pK_a values and differential solubility in organic solvents of these amines. It is well recognized that amines dissolve in acidic solutions and that their solubility can be maneuvered by altering the pH of the solution.[8] Nonetheless, this property

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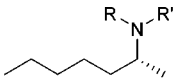

1 R=R'=H
2 R=H, R'= $CH_2C{\equiv}CH$
3 R=CH_3, R'= $CH_2C{\equiv}CH$
4 R=R'= $CH_2C{\equiv}CH$

Figure 1. **Structures of neuroprotective amines 2 and 3 under investigation, synthetic precursor (1) and byproduct (4).**

Table 1. **Qualitative experiments demonstrating partition of a secondary (2) and a tertiary amine (4) in 1.0 N NaH_2PO_3 buffer/hexanes system at various pH levels**

	presence of compound in the buffer	
pH	**2**	**4**
1.0	yes	yes
2.0	yes	trace
3.0	yes	no
4.0	yes	no

has not been widely studied for the separation of amines with varying degrees of substitution on N.[9] As the formation of amines with varying degrees of substitution is a common occurrence in a number of reactions,[1−3] this procedure has the potential to become a widely used tool in organic synthesis and more so for industrial process chemistry.

Systematic experiments were performed to optimize the conditions under which a mixture of amines with varying degrees of N-substitution as a solution in organic solvent can be selectively separated using multiple extractions with buffers of varying pH. As our interest was in optimizing a scalable process for preparing *R*-**2** and related compounds from corresponding primary amines, numerous experiments were designed and executed to study the various aspects of the purification process. For the detailed and repetitive process optimization investigation *racemic* **1** was used.

Our optimized reaction conditions to synthesize compound **2** from **1** produces a mixture containing **2** (∼64%), **4** (∼26%), and unreacted starting material **1** (∼10%) (Scheme 1).[5] To study the purification using buffer extractions, we decided to simplify our experiments by separately studying the dissolution behaviors of two two-component mixtures (**2** and **4** and **1** and **2**) initially and then apply the optimized purification procedure to the reaction mixture.

Table 1 summarizes our observation on the ability of NaH_2PO_3 buffer systems to selectively dissolve a particular amine from the hexanes solution of a mixture containing compounds **2** and **4** at varying pH values.

It is apparent that under strongly acidic conditions (pH ≈ 1.0), both **2** and **4** have appreciable solubility in the buffer, but as the pH of the buffer increases, its ability to trap **4** decreases much faster than it does for **2**. This may be explained by the increase in organic solubility of the more lipophilic amine at relative higher pH even if the environment is acidic. At pH 3.0 the presence of compound **4** in the buffer was undetectable on TLC, while compound **2** still had appreciable solubility. This clearly indicated that the two

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Table 2. **Quantitative experiments demonstrating partition of a secondary (2, 500 mg) and a tertiary amine (4, 500 mg) in 1.0 N NaH_2PO_3 buffer/hexanes system at various pH levels**

			calculated yield in mg (% yield)	
pH	recovery in buffer (mg)	**4:2** ratio by NMR	**2**	**4**
1.4	620	1:1	310 (62.2)	310 (62.0)
2.0	471	5.45:1	398 (79.6)	73 (14.6)
3.0	346	1:0	∼346 (∼69.2)	trace
4.0	312	1:0	312 (62.4)	−
5.0	306	1:0	306 (61.2)	−
6.0	121	1:0	121 (24.2)	−
7.0	19	1:0	19 (3.8)	−

Table 3. **Quantitative experiments demonstrating partition of a primary (1, 500 mg) and a secondary amine (2, 500 mg) in 1.0 N NaH_2PO_3 buffer/hexanes system at various pH levels**

			calculated yield in mg (% yield)	
pH	recovery in buffer (mg)	**1:2** ratio by NMR	**2**	**1**
6.0	395	∼4:1	80 (16)	315 (63)
6.5	313	12:1	25 (5)	288 (60)
7.0	175	∼50:1	trace	∼175 (∼35)
7.5	77	1:0	−	77 (15.4)
8.0	25	1:0	−	25 (5)
8.5	trace	1:0	−	trace

compounds can be selectively separated using this process. At this point, it was paramount to perform quantitative experiments under similar conditions to ascertain percent recovery at various pH levels. The results of such experiments are presented in Table 2 which also includes calculated amounts (based on the ^{1}H NMR spectrum) of the two components when the isolated material was still a mixture.

These results indicated that, while pH 3.0−4.0 would be ideal to remove compound **4** from the mixture, it will also be necessary to perform multiple extractions to have an acceptable level of compound **2** recovery.

On the basis of the pattern observed for the mixture of **2** and **4**, it was estimated that buffers of relatively higher pH will be required to trap compound **1** from a mixture containing **1** and **2**. This was indeed observed in the qualitative and quantitative experiments (cf. Experimental Section). According to the quantitative data (Table 3), pH 8.0 appeared optimum to safely remove compound **1** from this mixture, retaining **2** in the organic solution. Multiple extractions with buffer is indeed required to completely separate two compounds.

Experiments thus far were done with hexanes as the organic extraction solvent. It was reasonable to assume that the polarity of organic solvent may have considerable effect in the recovery/removal process particularly because lipophilicity of the amines appeared to play a vital role in the separation process. Thus, we decided to study the effects of common solvents in this process. Ethyl acetate, diethyl ether, dichloromethane, pentane, and hexanes were chosen as organic extraction solvents to optimize the recovery of compound **2** from the mixture containing **2** and **4** during the first extraction with NaH_2PO_3 buffer of pH 3.4 (previously

Table 4. **Quantitative experiments demonstrating partition of a secondary (2, 500 mg) and a tertiary amine (4, 500 mg) in 1.0 N NaH_2PO_3 buffer at pH 3.4 using various solvents**

organic solvent	**2** recovery (mg)	% recovery
ethyl acetate	273	54.6
diethyl ether	282	56.4
dichloromethane	309	61.8
pentane	341	68.2
hexanes	352	70.4

Table 5. **Quantitative experiments demonstrating partition of a secondary (2, 1000 mg) and a tertiary amine (4, 200 mg) using hexanes and NaH_2PO_3 buffer of various concentrations at pH 3.4**

concentration (N)	**2** recovery, g (%)	**4** recovery
0.5	0.36 (36)	0
1.0	0.424 (42.4)	0
1.5	0.449 (44.9)	0
2.0	0.459 (45.9)	0
2.5	0.554 (55.45)	0
3.0	0.57 (57.0)	trace

optimized for recovery of **2**; Table 2). Data obtained (Table 4) suggested that hexanes is the most suitable among the solvents studied for our purposes.

For the extraction-based purification process to be viable under industrial and/or small-scale laboratory setup, it is very important to regulate the volumes of extraction components. In this buffer-based extraction process, it was reasonable to believe that more concentrated buffer solution would be able to trap larger amount of compound **2** from the mixture containing **2** and **4** at pH 3.4 (optimized for NaH_2PO_3 buffer; Table 2). This was indeed reflected in the experiments carried out to verify the effect of buffer concentration, but the increase in recovery of **2** was not found to be linear with respect to buffer concentration (Table 5). Additionally, at higher concentration (e.g., 3.0 N) the buffer layer appeared to dissolve small amounts of compound **4**. This could be because of the "salt-out" effect at such a high concentration.

Another important variable in this process is the buffer itself; logically, different buffers may demonstrate different capacities to dissolve amines. In addition to the effect of NaH_2PO_3 buffer, those of two other buffers were tested for their ability to selectively dissolve compound **2** from the mixture containing **2** and **4** at pH 3.4. In qualitative experiments (cf. Experimental Section), it was found that, although various buffers can demonstrate the similar ability to partition the amines, the optimal pH for efficient separation may vary.

The KOH/citric acid buffer (B) was found to have a surprisingly larger capacity to selectively dissolve compound **2** than NaH_2PO_3 buffer (C) at pH 3.4. This experiment was then repeated twice to verify the consistency (Table 6). On the other hand, NaH_2PO_4 buffer (A) was found to be slightly inferior than C in bringing about the same effect. The most probable explanation of the superior performance of KOH/citric acid buffer is the use of organic acid which has a higher affinity for organic base.

Table 6. **Quantitative experiments demonstrating partition of a secondary (2, 1000 mg) and a tertiary amine (4, 200 mg) using hexanes and various buffers of 2.0 N concentration at pH 3.4**

buffer[a]	pH	**2** recovery,[c] mg (%)	**4** recovery[c] (mg)
A	3.4	370 (37)	0
B[b]	3.4	833 (83.3)	0
B[b]	3.4	870 (87.0)	0
B[b]	3.4	824 (82.4)	0
C	3.4	459 (45.9)	0

[a] A: NaH_2PO_4 buffer; B: KOH-citric acid buffer; C: NaH_2PO_3 buffer. For method of preparation, see Experimental Section. [b] This experiment was performed in triplicate. [c] Recovery after single extraction.

We also attempted to obtain similar results using H_3PO_3 acid solution of pH 3.0. It resulted in very poor selectivity, and effective separation was unsuccessful. This was expected as the pHs of acidic solutions change dramatically with additions of bases (e.g., amines) while buffers stabilize the pH.

One experiment was performed to find out how much of pure compound **2** can be recovered after multiple 1.0 N NaH_2PO_3 buffer extractions at pH 3.4 from a mixture of known composition of compounds **2** and **4**. After five extractions up to 90.5% of **2** was recovered in pure form. This shows that the recovery is reasonably good.

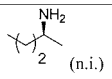
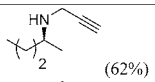
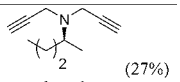
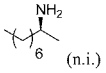
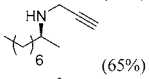
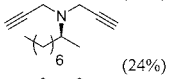
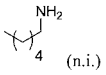
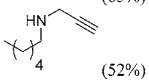
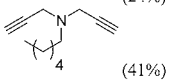
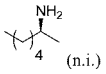
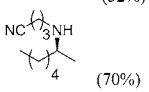
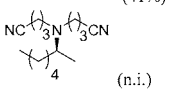
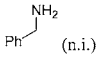
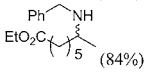
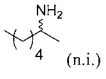
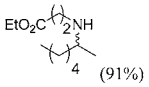
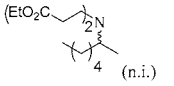
Also, we modified our procedure to prepare compound **2** in large scale where our newly developed buffer-based purification process can be used. In one of the trial runs, we were able to selectively separate compound **2** in ~63% yield. Finer purification by distillation can then be performed on this material.

This process appears to be fairly robust and of wide application. Since our drug discovery program involved synthesis of analogues of compound **2**, we were using similar chemistry to produce these analogues. Thus, we had a number of reaction mixtures available on which to test our process. We could successfully demonstrate qualitatively that this purification procedure indeed led to successful separation of the constituent amines with reasonable purity (Mixtures **A**−**F**; Table 7); we had to adjust the pH values in two cases. In all the cases, mono-N-substituted products were target compounds to be isolated. NaH_2PO_3 buffer (1.0 N) with pH 3.0 was applied to get rid of the di-N-substituted compound, while the buffer with pH 8.0 was used to remove the primary amine. In the cases of mixtures **C** and **E**, buffer with pH 7.0 worked better to remove corresponding primary amines.

Conclusion

We have demonstrated that a mixture of primary, secondary, and tertiary amines can be successfully separated by a buffer-based extraction procedure. This procedure can routinely be employed in day to day laboratory practice, and after a small amount of optimization, time-consuming column chromatography can be avoided. This process of amine purification has the potential to find wide application in industrial purification processes.

Table 7. **Mixtures successfully separated by buffer extraction[a]**

Mixture	Components		
	1	**2** (% Yield)	**3** (% Yield)
A	 (n.i.)	 (62%)	 (27%)
B	 (n.i.)	 (65%)	 (24%)
C	 (n.i.)	 (52%)	 (41%)
D	 (n.i.)	 (70%)	 (n.i.)
E	 (n.i.)	 (84%)	
F	 (n.i.)	 (91%)	 (n.i.)

[a] n.i.: Not isolated.

Experimental Methods

Unless otherwise stated, ^{1}H NMR spectra were recorded in deuterated chloroform on a Bruker AMX500 spectrometer operating at 500 MHz. Chemical shifts are expressed in parts per million (ppm) relative to TMS. Chemicals and solvents were purchased from Aldrich Chemical Co. without further purification. Chek-Mite pH-15 pH meter was used for pH measurements after proper calibrations.

Preparation of NaH_2PO_3 Buffer Solution of Different pH Values.[10] NaH_2PO_3 buffer (1.0 N) was prepared by mixing equal volumes of 2.0 N aq NaOH (8.0 g in 100 mL) and aq 4.0 N H_3PO_3 (16.4 g in 100 mL). The buffers with different pHs were obtained by either adding aq H_3PO_4 or aq NaOH to adjust the pH. In this way, buffers with pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 were prepared.

1. The Solubility of Compounds 2 and 4 in Buffers of Different pH Value. *a. Qualitative Experiments.* A 1:1 mixture containing **2** and **4** (50 mg) was mixed with appropriate buffer (5 mL) and hexanes (5 mL) in a separatory funnel, and it was shaken vigorously. The aqueous layer was separated, basified with 15% aq NaOH to pH $\approx$ 12, and finally partitioned with 5 mL of hexanes. TLC was performed on the two organic layers. The results are shown in Table 1.

b. Quantitative Experiments. Compounds **2** (0.5 g) and **4** (0.5 g) were dissolved in hexanes (20 mL) and then extracted with 1.0 N NaH_2PO_3 buffer (20 mL) of appropriate pH. The organic layer was discarded, and the aqueous layer was basified with 15% aq NaOH and extracted with diethyl ether (30 mL $\times$ 2). The combined ether solution was dried over anhydrous $MgSO_4$, filtered, and rotary evaporated to dryness. In case a mixture was obtained, the ratio of **2** and **4** was analyzed by ^{1}H NMR. The results are displayed in Table 2.

2. The Solubility of Compounds 1 and 2 in Buffers of Different pH Values. *a. Qualitative Experiments.* Compounds **1** and **2** (100 mg each) were dissolved in hexanes (20 mL) to prepare the stock solution. Extractions were carried out on this solution (2 mL for each experiment) using buffers (2 mL) of varying pH (5.0, 6.0, 7.0, 8.0, 9.0, and 10.0). The two layers were separated. The aqueous layer was basified with 15% aqueous NaOH to pH $\approx$ 12, and finally extracted with 5 mL of hexanes. TLCs were performed on both the layers of hexanes. Buffers with pH > 8.0 were found to retain compound **1** only while the organic layer was found to be enriched in **2**. Multiple extractions of the organic layer with the buffer (pH 8.0) led to purification of **2**.

b. Quantitative Experiments. Compounds **1** (0.5 g) and **2** (0.5 g) were mixed and dissolved in hexanes (20 mL) and then extracted with buffer (20 mL) of varying pH. The resulting buffer solution was separated and basified to pH $\approx$ 11 with 15% NaOH, which was extracted with diethyl ether (30 mL $\times$ 2). The ether layer was dried over anhydrous $MgSO_4$ and rotary evaporated to dryness. In case a mixture was obtained, the ratio of **1** and **2** was calculated on the basis of NMR. The results obtained are indicated in Table 3.

3. Effect of Organic Extraction Solvents. Compounds **2** (0.5 g) and **4** (0.5 g) were mixed and dissolved in various solvents (20 mL) and extracted (once) with 1.0 N NaH_2PO_3 buffer (20 mL; pH 3.4). The buffer layer was separated, basified to pH 11.0, and extracted with diethyl ether (10 mL $\times$ 2). The ether solution was dried over anhydrous $MgSO_4$ and rotary evaporated. The residue was identified by TLC to be **2** only. Table 4 indicates the recovery of **2** from different organic solutions.

4. The Concentration of the Buffer vs Recovery. NaH_2PO_3 buffers were made in varying concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 N, pH $\approx$ 3.4) by mixing aq NaOH and aq H_3PO_3 solutions of appropriate concentrations. Compounds **2** (1.0 g) and **4** (0.2 g) were dissolved in hexanes (10 mL), and a buffer solution (10 mL) with different concentrations was added and mixed well. The buffer layer was separated and basified with 15% aq NaOH to pH $\approx$ 12.0 and extracted with diethyl either (20 mL $\times$ 2). The combined ether solution was dried over anhydrous $MgSO_4$ and evaporated to give products. The 5:1 (w/w) composition of the two compounds was used to simulate the N-propargylation reaction product mixture. Table 5 contains the results of these experiments.

5. Effect of Different Buffers. *Buffer A (2.0 N NaH_2PO_4 Buffer).*[10] Equal volumes of 2.0 N aq NaOH (8 g in 100 mL) and 6.0 N aq H_3PO_4 (19.6 g in 100 mL) were mixed. The pH was then adjusted to 3.0, 3.4, and 8.0 by adding either aq H_3PO_3 or aq NaOH.

Buffer B (2.0 N KOH−Citric Acid Buffer).[10] Equal volumes of 2.0 N aq KOH (12.4 g in 100 mL, assuming KOH pallets to be 90% based on label information) and 6.0 N aq citric acid (38.4 g in 100 mL). The pH was then adjusted to 3.0, 3.4, 4.0, 7.0, and 8.0, adding either citric acid powder or aq NaOH.

Buffer C (1.0 N NaH_2PO_3 Buffer).[10] Prepared as mentioned before in this section.

(10) Phosphoric acid (H_3PO_4) and citric acid are tribasic acid, while phosphorus acid (H_3PO_3) is dibasic. In each case of buffer preparation, only one acidic proton reacts with the base, leaving NaOH/H_3PO_4 and KOH/citric acid buffers dibasic and NaOH/H_3PO_3 buffer monobasic.

a. Qualitative Experiments. A mixture of **1**, **2**, and **4** (100 mg each) was dissolved in hexanes (4 mL) and partitioned in buffer (4 mL) with lower pH. The buffer layer was basified and extracted with hexanes (4 mL). This organic layer was washed with buffer (4 mL) with higher pH. TLC was performed to the final hexanes layer. Only compound **2** was detected in the final hexanes solution. For experiments with buffer **A**, pH 3.0 was found to be optimum to remove **4**, while that with pH 8.0 was most favorable to remove **1**; in case of buffer **B**, these values were 4.0 and 7.0, respectively.

b. Quantitative Experiments. Compounds **2** (1.0 g) and **4** (0.2 g) (ratio chosen to simulate the product composition after N-propargylation reaction[5] on compound **1**) were dissolved in hexanes (10 mL), and appropriate buffer (pH 3.4, 10 mL) was added to this solution. This mixture was shaken violently in a separatory funnel. The buffer layer was separated and basified with 15% aq NaOH to pH $\approx$ 11.0. After extraction with diethyl ether (10 mL $\times$ 2) and drying over anhydrous $MgSO_4$, the filtrate was evaporated to retrieve compound **2**. Table 6 contains the results of these experiments.

6. Separation of Amine Mixtures with Aqueous Acid Solutions. A mixture of **2** (50 mg) and **4** (50 mg) was added to aq H_3PO_3 solution (5 mL, pH 3.0). It was then extracted with hexanes; the aq layer was basified with 15% aq NaOH. The basified layer was again extracted with hexanes (3 mL). The TLC of the final hexanes layer showed the presence of **2** only, but the recovery after rotary evaporation was found to be extremely low ($\sim$3%).

7. Optimization of Recovery by Repeated Extractions. A mixture of **2** (916 mg) and **4** (410 mg) was dissolved in hexanes (20 mL) and partitioned with 1.0 N NaH_2PO_3 buffer (5 $\times$ 15 mL, pH 3.4). The combined buffer solution was basified with 15% aq NaOH and then extracted with hexanes (2 $\times$ 60 mL). The final hexanes layer was dried over anhydrous $MgSO_4$ and evaporated to obtain **2** in 90.5% yield (829 mg).

8. Synthesis and Buffer-Assisted Purification of Compound 2. To a two-phase solution of **1** (2.875 g, 25 mmol) in diethyl ether (25 mL) and 10% aq Na_2CO_3 (40 mL) was added propargyl benzenesulfonate (5.558 g, 27.5 mmol) dropwise at room temperature and then was stirred at 40 °C for 18 h. The reaction mixture was cooled to room temperature. The organic layer was separated, and the aqueous layer was extracted with hexanes (2 $\times$ 50 mL). The ether solution and the hexanes extractions were combined and filtered, and the filtrate was rotary evaporated to dryness. The residue was dissolved in hexanes (60 mL). The organic solution was extracted with 1.0 N NaH_2PO_3 buffer (pH = 3.4; 4 $\times$ 30 mL), and the organic layer was checked by TLC for presence of **2** (only very trace amounts were detected). The buffer extractions were combined and washed with hexanes (10 mL). This hexanes layer was discarded. The buffer extractions were combined and basified to pH $\approx$ 12 with 15% aq NaOH, and this was extracted with diethyl ether (3 $\times$ 50 mL). The combined ether extractions were washed with buffer of pH 8.0 twice to remove any remaining **1**. The ether solution was then dried over anhydrous $MgSO_4$, filtered, and evaporated to give **2** as clear or slightly yellowish oil (2.396 g, yield 62.6%).

9. General Procedure for Alkylation of Compounds A−D and Buffer-Assisted Purification of the Alkylated Products. To a two-phase solution of the primary amine- (**A**−**D**, 25 mmol) in diethyl ether (25 mL) and 10% aq Na_2CO_3 (40 mL) was added the alkylating agent (corresponding bromide, 27.5 mmol) dropwise at room temperature, and then the mixture was stirred at 40 °C for 18 h. The reaction mixture was cooled to room temperature. The organic layer was separated, and the aqueous layer was extracted with hexanes (2 $\times$ 50 mL). The ether solution and the hexanes extractions were combined and filtered, and the filtrate was rotary evaporated to dryness.

The residue was dissolved in hexanes (60 mL). The organic solution was extracted with 1.0 N NaH_2PO_3 buffer (lower pH 3.0 or 3.4; 4 $\times$ 30 mL). The buffer extractions were combined and washed with hexanes (10 mL). All the hexanes layers were combined and checked by TLC. It mainly contained the tertiary amine product with just a trace of the secondary amine product. Another buffer extraction (lower pH 3.0 or 3.4; 4 $\times$ 30 mL) eliminated the secondary amine almost completely from the organic layer. The evaporation of the organic layer furnished crude tertiary amine product (**A3**−**D3**). The buffer extractions were combined and basified to pH $\approx$ 12 with 15% aq NaOH and was extracted with diethyl ether (3 $\times$ 50 mL). The combined ether extractions were washed with buffer of higher pH (7.0− 8.0) twice to remove any remaining primary amine. The ether solution was then dried over anhydrous $MgSO_4$, filtered, and evaporated to give the secondary amine product (**A2**−**D2**).

Reductive amination of ethyl 7-oxooctanoate with benzylamine (**E1**) following a related literature procedure[11] produced a mixture of **E2** and **E1**. Mixture of **F1**−**F3** was obtained by Michael-type addition[12] of ethyl acrylate on **F1**. The buffer-assisted purification of these mixtures was performed in a similar manner as described above.

(S)-N-(Prop-2-ynyl)pentan-2-amine (A2): Characterized after conversion to HCl salt. Mp 111.9−112.6 °C. ^{1}H NMR (500 MHz, D_2O): δ 0.82 (3H, t, J = 7.4 Hz, 5-CH_3), 1.20 (3H, t, J = 6.7 Hz, 1-CH_3), 1.21−1.38 (2H, m, 4-CH_2), 1.40−1.65 (2H, m, 3-CH_2), 2.85 (1H, t, J = 2.5 Hz, ≡CH), 3.33−3.40 (1H, m, 2-CH), 3.79−3.88 (2H, m, NCH_2). FAB MS (70 eV): m/z 126 (M + 1, 100).

(S)-N,N-Di(prop-2-ynyl)pentan-2-amine (A3): Characterized after conversion to HCl salt. Mp 177.4−178.5 °C. ^{1}H NMR (500 MHz, D_2O): δ 0.84 (3H, t, J = 7.2 Hz, 5-CH_3), 1.27−1.40 (5H, m, 4-CH_2, 1-CH_3), 1.49−1.70 (2H, m, 3-CH_2), 3.00 (2H, t, J = 2.3 Hz, 2 $\times$ ≡CH), 3.73−3.80 (1H, m, 2-CH), 4.05−4.15 (4H, m, 2 $\times$ NCH_2).

(S)-N-(Prop-2-ynyl)nonan-2-amine (B2): Characterized after conversion to HCl salt. Mp 89.3−90.6 °C. ^{1}H NMR (500 MHz, D_2O): δ 0.76 (3H, t, J = 7.4 Hz, 9-CH_3), 1.13− 1.33 (13H, m, 1-CH_3, 5 $\times$ −CH_2), 1.41−1.67 (2H, m, 3-CH_2), 2.86 (1H, t, J = 2.6 Hz, ≡CH), 3.31−3.38 (1H, m,

(11) Kennedy, K. J.; Lundquist, J. T.; Simandan, T. L.; Beeson, C. C.; Dix, T. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1937−1940.
(12) Stork, G.; McElvain, S. M. *J. Am. Chem. Soc.* **1947**, *69*, 971−972.

2-CH), 3.78−3.87 (2H, m, NCH_2); FAB MS (70 eV): *m*/*z* 182 (M + 1, 100).

(S)-N,N-Di(prop-2-ynyl)nonan-2-amine (B3): Characterized after conversion to HCl salt. Mp 84.2−84.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (3H, t, J = 7.9 Hz, 9-CH_3), 1.05 (3H, d, J = 6.5 Hz, 1-CH_3), 1.20−1.34 (10H, m, 5 × $-CH_2$), 1.49−1.58 (2H, m, 3-CH_2), 2.17 (2H, t, J = 2.4 Hz, 2 × ≡CH), 2.77−2.84 (1H, m, 2-CH), 3.43−3.53 (4H, m, 2 × NCH_2). FAB MS (70 eV): *m*/*z* 220 (M + 1, 100).

N-(Prop-2-ynyl)hexan-1-amine (C2): Characterized after conversion to HCl salt. Mp 168.3−169.3 °C. ^{1}H NMR (500 MHz, D_2O): δ 0.76 (3H, t, J = 6.8 Hz, 6-CH_3), 1.15−1.30 (6H, m, 3 × $-CH_2$), 1.52−1.62 (2H, m, 2-CH_2), 2.86 (1H, m, ≡CH), 3.03 (2H, t, J = 7.3 Hz, 1-CH_2), 3.79 (2H, bs, NCH_2).

N,N-Di(prop-2-ynyl)hexan-1-amine (C3): ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (3H, t, J = 7.0 Hz, 6-CH_3), 1.22−0.132 (6H, m, 3 × $-CH_2$), 1.40−1.49 (2H, m, 2-CH_2), 2.19 (2H, t, J = 2.4 Hz, 2 × ≡CH), 2.49 (2H, t, J = 7.5 Hz, 1-CH_2), 3.41 (4H, d, J = 2.4 Hz, 2 × NCH_2).

3-((S)-Heptan-2-ylamino)butanenitrile (D2): mp 77.3−77.4 °C (HCl salt). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (3H, t, J = 7.1 Hz, 7-CH_3), 0.98 (3H, d, J = 6.3 Hz, 1-CH_3), 1.18−1.40 (8H, m, 4 × CH_2), 1.70−1.80 (2H, m, $-NCH_2$-CH_2), 2.41 (2H, t, J = 7.1, CH_2CN), 2.52−2.60 (1H, m, 2-CH), 2.60−2.68 (2H, m, NCH_2).

Ethyl 7-(benzylamino)octanoate (E2): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (3H, t, J = 6.3 Hz, 8-CH_3), 1.13 (1H, bs, NH), 1.22 (3H, t, J = 7.1 Hz, $-OCH_2CH_3$), 1.25−1.35 (4H, m, 2 × $-CH_2$), 1.42−1.47 (2H, m, 6-CH_2), 1.55−1.64 (2H, m, 3-CH_2), 2.25 (2H, t, J = 7.6, $COCH_2$), 2.60−2.66 (1H, m, 2-CH). 3.75 (2H, dd, J = 32.7, 12.9 Hz, Ar−CH_2), 4.09 (2H, q, J = 7.1, OCH_2), 7.19−7.30 (5H, m, Ar−H).

Ethyl 3-(heptan-2-ylamino)propanoate (F2): ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (3H, t, J = 7.2 Hz, 7-CH_3), 1.00 (3H, d, J = 6.3 Hz, 1-CH_3), 1.15−1.29 (9H, m, 3 × CH_2, $-OCH_2CH_3$), 1.38−1.50 (3H, m, 3-CH_2, NH) 2.46 (2H, t, J = 6.0, $COCH_2$), 2.77−2.90 (2H, m, NCH_2), 2.55−2.61 (1H, m, 2-CH), 4.11 (2H, q, J = 7.1, OCH_2). FAB MS (70 eV): *m*/*z* 216 (M + 1, 100).

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