JOURNAL OF combinatorial CHEMISTRY



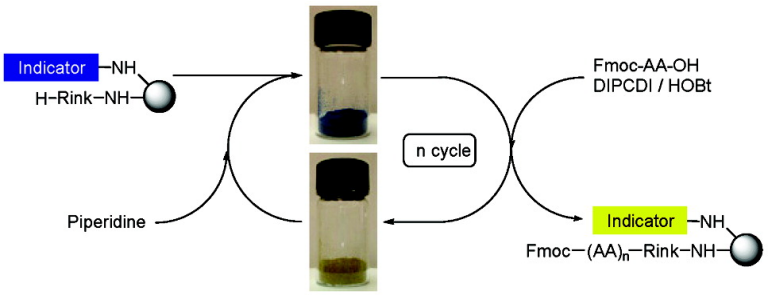
Article

# Self-Indicating Resins: Sensor Beads and in Situ Reaction Monitoring

Jin K. Cho, Peter D. White, Wolfgang Klute, Tony W. Dean, and Mark Bradley

*J. Comb. Chem.*, **2003**, 5 (5), 632-636• DOI: 10.1021/cc034010i • Publication Date (Web): 12 July 2003






## More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

# Self-Indicating Resins: Sensor Beads and in Situ Reaction Monitoring

Jin K. Cho,[†] Peter D. White,[‡] Wolfgang Klute,[§] Tony W. Dean,[⊥] and Mark Bradley*[,†]

*Combinatorial Centre of Excellence, Department of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, U.K., Merck Biosciences Ltd., Boulevard Industrial Park, Padge Road, Beeston, Nottingham NG9 2JR, U.K., Pfizer Global Research & Development, Ramsgate Road, Sandwich CT13 9NJ, U.K., and GlaxoSmithKline, Discovery Research, Gunnels Wood Road, Stevenage SG1 2NY, U.K.*

*Received May 22, 2003*

Solid-phase organic synthesis and solution-phase parallel synthesis assisted by polymer-supported reagents, or scavenger resins, enable painstaking purifications to be avoided during synthesis. This concept was applied to reaction monitoring through the development of resin-bound indicators for the efficient analysis of various chemistries. Bromophenol blue was chosen as the indicator and attached to the resin after derivatization. The resin was successfully used as a "sensor" for monitoring solid-phase peptide synthesis, and applied for in situ reaction monitoring during the synthesis of a library of ureas in a highly successful manner.

Combinatorial strategies often employ a variety of resin-based technologies to improve synthetic efficiency. This includes the use of solid-phase organic synthesis as well as the application of polymer-supported reagents and quenching agents.[1] One of the many advantages of using resin-bound systems is that chemistry can be mediated without inflicting painstaking purification procedures on the synthetic chemist, something that is of crucial importance when arrays of compounds are being generated. One issue that challenges the synthetic chemist when using solid-phase chemistry is that of reaction analysis, an issue that is complicated when it becomes necessary to monitor multiple reactions in an array-type format. Several colorimetric methods have been developed to analyze reactions on a variety of solid supports,[2] the most widely used being the ninhydrin test,[3] first introduced by Kaiser in 1970 to enable the completion of peptide coupling reactions to be monitored, although requiring the destruction of a small sample of resin for each assay and extensive time. Numerous other tests based on this philosophy have since been introduced,[4] but the ninhydrin test remains the stalwart of the solid-phase peptide chemist. Other tests have been introduced that allow real-time monitoring. This includes continuous flow peptide synthesis and the monitoring of the release of the Fmoc protecting group,[5] the use of Dhbt-based active esters, and direct resin monitoring,[6] as well as the use of highly controlled amounts of bromophenol blue to monitor in situ peptide coupling.[7] This method is especially attractive because of the highly visible color changes that take place during the reaction process, but does require a certain amount of experimentation to ensure the correct amount of dye is added.

* To whom correspondence should be addressed. Phone: 44 23 8059 3598. Fax: 44 23 8059 6766. E-mail: mb14@soton.ac.uk.
[†] University of Southampton.
[‡] Merck Biosciences Ltd.
[§] Pfizer Global Research & Development.
[⊥] GlaxoSmithKline.

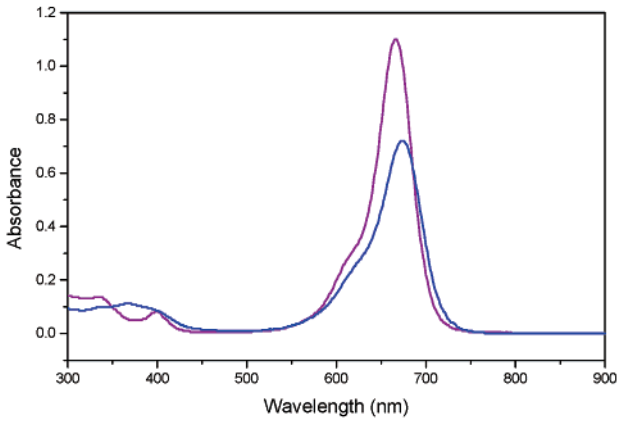
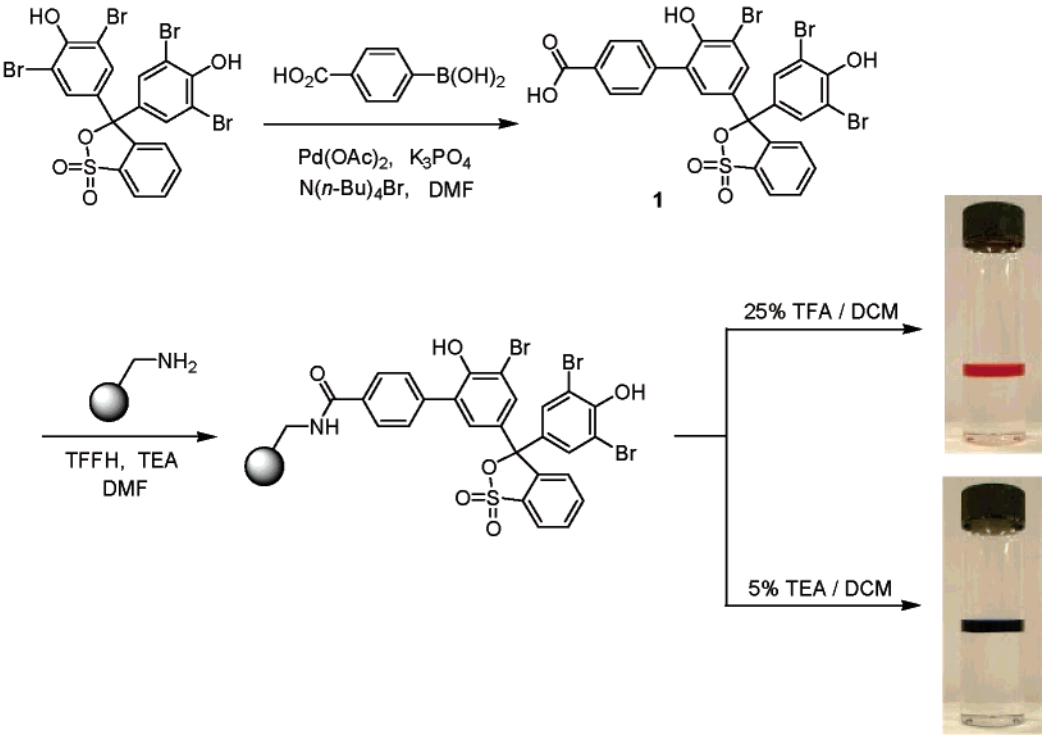



**Figure 1.** UV/vis spectra of 10 $\mu$M bromophenol blue (purple line) and 10 $\mu$M bromophenol blue derivative **1** (blue line), both in the presence of 20 $\mu$M piperidine in DMF.

Here we describe a new concept for solid-phase synthesis, that of "self-indicating" resins in which resin-bound indicators act as integral sensors for the chemistry being carried out, either directly on the sensor bead itself or as a probe for solution chemistry taking place around it, thus allowing direct in situ monitoring of the chemistry being undertaken. The dye bromophenol blue (3′,3″,5′,5″-tetrabromophenolsulfophthalein) was chosen as an indicator in consideration of the conspicuous color change from yellow to dark blue and its high extinction coefficient, thus giving high sensitivity. To allow immobilization onto the resin without loss of conjugation, a carboxylic acid group was introduced onto bromophenol blue via a Suzuki cross-coupling reaction of bromophenol blue with 4-carboxyphenylboronic acid, allowing coupling to a number of solid supports.[8] Importantly, the absorption spectra of compound **1** compared well with that of bromophenol blue. $\lambda_{max}$ slightly increased from 603 to 610 nm, while $\epsilon_{max}$ decreased from 110 000 to 73 000 (Figure 1).

10.1021/cc034010i CCC: $25.00 
Published on Web 07/12/2003

**Scheme 1.** Preparation of Resin-Bound Indicator




Compound **1** was initially attached to aminomethyl NovaGel resin using tetramethylfluoroformamidinium hexafluorophosphate (TFFH) as a coupling agent.[9] The resin appeared red in acidic solution and dark blue in basic solution, as anticipated (Scheme 1).

The self-indicating resin was used as an internal sensor. Five percent of sites on the resin beads (0.74 mmol of $NH_2$/g) were loaded with dye **1**, and the resin was then divided into two. One portion was capped with acetic anhydride (negative Ninhydrin test), and the other portion was left uncapped. When treated with DMF, the color of the capped resin did not change, but the resin with free amino groups turned blue as a result of intrabead interactions. Fmoc-Leu-OH was coupled onto the self-indicating resin using DIPCDI/HOBt, and samples were taken for quantitative analysis. The resin remained blue until 98% of the sites had been coupled, whereupon it appeared green and then greenish yellow (a quantitative ninhydrin test showed that >99% of amino groups on the resin were acylated when the resin was greenish yellow). With this preliminary test of success, the self-indicating resin was used as an in situ indicator for solid-phase peptide synthesis. Thus, 5% of the total amino groups of aminomethyl NovaGel resin were coupled to **1**, and the remaining amino groups were coupled with the Fmoc-Rink linker using DIPCDI/HOBt. Leu-enkephalinamide (H−Tyr-Gly-Gly-Phe-Leu−$NH_2$), and the more difficult sequence (65−74 of acyl carrier protein, H−Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly−$NH_2$) were synthesized. Couplings were mediated by DIPCDI/HOBt, and the completion of coupling was detected via a color change from blue to greenish yellow. No other monitoring method was employed or necessary during the synthesis of the peptide. The coupling of certain residues was difficult, and in these cases, prolonged reaction times or double couplings were required for completion of the color change[10] (these coincided with previous observations).[11] Leu-enkephalinamide was prepared in 96% yield, 98% purity. 65−74 of acyl carrier protein was prepared in 92% yield and 89% purity (Figure 2).[12]

The self-indicating resin was used for the in-situ monitoring of polymer-supported purification for parallel solution-phase synthesis,[13] useful, since parallel synthesis requires simple and convenient tools to determine the completion of each reaction in an array. In this regard, the resin-bound indicator avoids the need for laborious conventional chromatographic analysis. The sensitivity of the resin-bound indicator was investigated by looking at various concentrations of benzylamine in solution, and the resin-bound indicator (5%) could clearly detect concentrations of benzylamine down to 100 $\mu$M. A library of ureas was prepared using six amines (0.25 mmol each) and four isocyanates (0.2 mmol each) as building blocks. When the self-indicating resin was added to each well after urea formation, the color of the resin immediately changed to blue as a result of excess amine. When methylisocyanate resin (100 mg each, 1.6 mmol/g resin) was added as an amine scavenger, the color of the self-indicating resins gradually turned to yellow to give crude products with purities of 93−99% (Figure 3, Table 1).

In summary, resin-bound indicators allow the detection of amines both in solution and on the solid phase. The resin-bound dye can be used as a self-indicator for in situ monitoring of solid-phase peptide synthesis as well as array library synthesis. Since the newly developed self-indicating resins are able to follow coupling reactions at the level of individual beads (instead of checking reaction completion globally), this resin should be helpful during mix-and-split synthesis in which every bead may contain a different peptide sequence and therefore exhibit different reaction kinetics. It also offers the possibility of monitoring the release of free amines on the resin during chemical or biological screening.

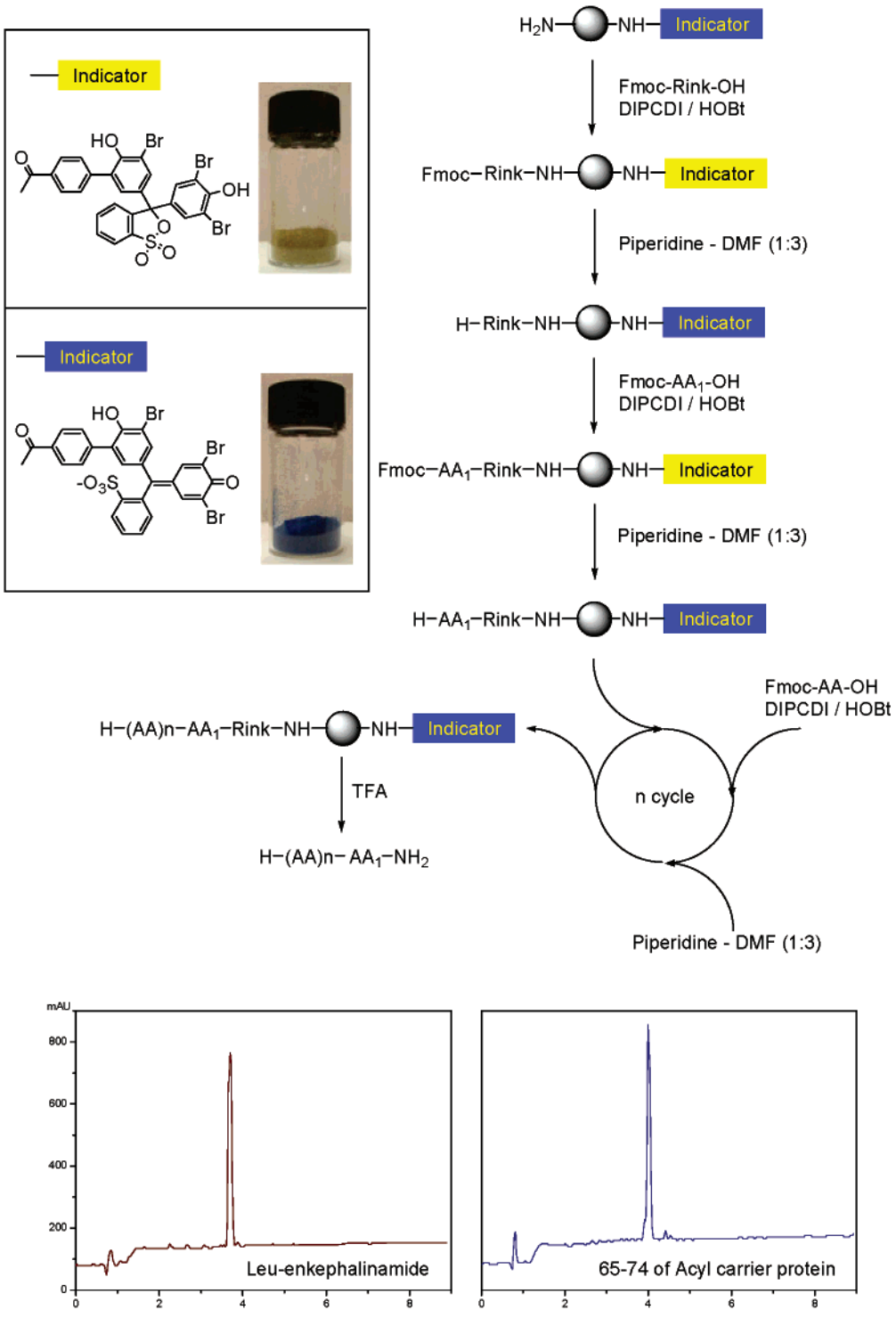



**Figure 2.** Solid-phase peptide synthesis on self-indicating resin.

## Experimental Details

**Preparation of Compound 1.** In a tube reactor were placed bromophenol blue (200 mg, 0.3 mmol), 4-carboxyphenylboronic acid (0.36 mmol, 1.2 equiv), potassium phosphate (0.9 mmol, 3.0 equiv), tetrabutylammonium bromide (1.0 mol %), and palladium(II) acetate (2.0 mol %). The reactor was capped with a septum and gently sparged with argon for 10 min. DMF (3 mL) was added through a septum, and the reaction mixture was stirred under argon at 110 °C for 18 h. The resulting mixture was treated with saturated $KHSO_4$, extracted with EtOAc, dried over $Na_2SO_4$, and evaporated under reduced pressure to afford a reddish oil. The product was isolated by column chromatography on silica gel (chloroform/methanol/acetic acid = 5:1:0.06) to give **1** in 30% yield.[8]

**Self-Indicating Resin (5% Indicator-Containing Fmoc-Rink-Resin) for Solid-Phase Peptide Synthesis.** Compound **1** (0.004 mmol, 0.05 equiv) and triethylamine (0.012 mmol, 0.15 equiv) dissolved in DMF (2 mL) were added to the aminomethyl NovaGel resin (100 mg, 0.074 mmol, 1.0 equiv) and premixed for 10 min. TFFH (0.006 mmol, 0.06 equiv) dissolved in DMF (1 mL) was slowly added to the

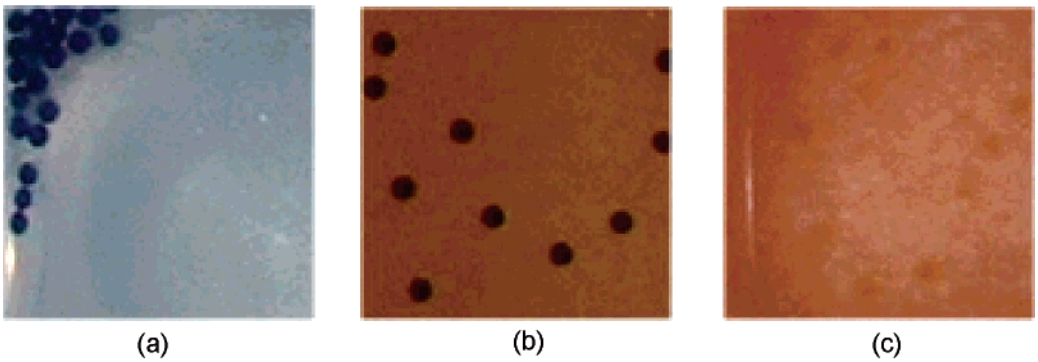
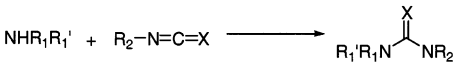



**Figure 3.** Color change of the self-indicating resin as the amine is scavenged by the methylisocyanate resin: (a) before scavenger resin added, (b) as soon as scavenger resin is added, (c) after amines in solution were completely scavenged.

**Table 1.** Summary of Urea Formation Library




| entry | $R_1$, $R_1'$ | $R_2$ | X | yield | purity |
|---|---|---|---|---|---|
| 1 | $CH_3(CH_2)_2CH_2$, H | $PhCH_2$ | O | 100 | 97 |
| 2 | $CH_3CH(CH_3)CH_2CH_2$, H | $PhCH_2$ | O | 100 | 98 |
| 3 | $PhCH_2$, H | $PhCH_2$ | O | 86 | 99 |
| 4 | 4-$CH_3OPhCH_2$, H | $PhCH_2$ | O | 99 | 99 |
| 5 | 4-$CF_3PhCH_2$, H | $PhCH_2$ | O | 76 | 97 |
| 6 | $CH_2(CH_2)_3CH_2$ | $PhCH_2$ | O | 100 | 99 |
| 7 | $CH_3(CH_2)_2CH_2$, H | 4-$CH_3OPhCH_2$ | O | 94 | 98 |
| 8 | $CH_3CH(CH_3)CH_2CH_2$, H | 4-$CH_3OPhCH_2$ | O | 100 | 97 |
| 9 | $PhCH_2$, H | 4-$CH_3OPhCH_2$ | O | 97 | 99 |
| 10 | 4-$CH_3OPhCH_2$, H | 4-$CH_3OPhCH_2$ | O | 32 | 94 |
| 11 | 4-$CF_3PhCH_2$, H | 4-$CH_3OPhCH_2$ | O | 60 | 95 |
| 12 | $CH_2(CH_2)_3CH_2$ | 4-$CH_3OPhCH_2$ | O | 100 | 98 |
| 13 | $CH_3(CH_2)_2CH_2$, H | 2-$ClPhCH_2$ | O | 100 | 96 |
| 14 | $CH_3CH(CH_3)CH_2CH_2$, H | 2-$ClPhCH_2$ | O | 100 | 95 |
| 15 | $PhCH_2$, H | 2-$ClPhCH_2$ | O | 46 | 93 |
| 16 | 4-$CH_3OPhCH_2$, H | 2-$ClPhCH_2$ | O | 73 | 96 |
| 17 | 4-$CF_3PhCH_2$, H | 2-$ClPhCH_2$ | O | 94 | 98 |
| 18 | $CH_2(CH_2)_3CH_2$ | 2-$ClPhCH_2$ | O | 100 | 97 |
| 19 | $CH_3(CH_2)_2CH_2$, H | $PhCH_2$ | S | 100 | 98 |
| 20 | $CH_3CH(CH_3)CH_2CH_2$, H | $PhCH_2$ | S | 100 | 97 |
| 21 | $PhCH_2$, H | $PhCH_2$ | S | 86 | 93 |
| 22 | 4-$CH_3OPhCH_2$, H | $PhCH_2$ | S | 100 | 95 |
| 23 | 4-$CF_3PhCH_2$, H | $PhCH_2$ | S | 81 | 97 |
| 24 | $CH_2(CH_2)_3CH_2$ | $PhCH_2$ | S | 100 | 99 |

suspension, and the reaction mixture was stirred for 4 h. The resins were washed with 25% TFA in DCM and 5% TEA in DCM in turns until no blue color bleached out. The remaining amino groups were coupled with Fmoc-Rink-OH (0.22 mmol, 3.0 equiv) using DIPCDI (0.22 mmol, 3.0 equiv) and HOBt (0.22 mmol, 3.0 equiv) in DMF (3 mL).

**Solid-Phase Peptide Synthesis on Self-Indicating Resin.** 5% indicator-containing Fmoc-Rink-resin (100 mg, 0.04 mmol) was treated with 20% piperidine in DMF (3 mL each, 3 + 17 min). For each cycle, preactivated Fmoc-amino acids (0.12 mmol, 3.0 equiv) with DIPCDI (0.12 mmol, 3.0 equiv) and HOBt (0.12 mmol, 3.0 equiv) in DMF (3 mL) were added and shaken until the blue color of the resin disappeared. Aspartic acid and tyrosine were protected as their *tert*-butyl ester and ether, respectively. Asparagine and glutamine were protected as their trityl derivatives. All washings after couplings and deprotections were performed with DCM. Cleavages of peptides from the resins were achieved with 90% TFA in DCM (3 mL) for 3 h, and the cleaved resins were washed with additional 90% TFA in DCM. The combined filtrates were evaporated (nitrogen bubbling), dissolved in water, and lyophilized. The cleaved yields were 96% for leucine-enkephalinamide and 98% for 65−74 of acyl carrier protein on the basis of Fmoc quantification after coupling of Fmoc-Rink-OH. Leucine-enkephalinamide has a calculated mass of 554.3. ESI-MS, *m/z*, positive $[M + H]^+$, 555.2. 65−74 of acyl carrier protein has a calculated mass of 1061.6. ESI-MS, *m/z*, positive $[M + H]^+$, 1062.2.

**Self-Indicating Resin for Urea Library.** Compund **1** (0.05 mmol, 0.1 equiv) and triethylamine (0.15 mmol, 0.3 equiv) dissolved in DMF (5 mL) were added to the aminomethylated polystyrene resin (500 mg, 0.50 mmol, 1.0 equiv, ∼0.4 to 0.5 mm diameter) and premixed for 10 min. TFFH (0.06, 0.12 equiv) dissolved in DMF (1 mL) was slowly added to the suspended solution, and the reaction mixture was stirred for 4 h. The resins were washed with 25% TFA in DCM and 5% TEA in DCM by turns until no blue color bleached out. The remaining amino groups of the resulting resin were completely acetylated with acetic anhydride (5.0 mmol, 10 equiv) and triethylamine (5.0 mmol, 10 equiv) in DCM (5 mL).

**Urea Library.** In each column (4 wells) of a 24-well microplate (6 × 4 wells) were placed six amines (0.25 mmol, 1.25 equiv) in dry DCM (3 mL). Three isocyanates and one isothiocyanate (0.2 mmol, 1.0 equiv) were added to each row (6 wells), and the microplate was shaken for 2 h. Self-indicating resin (∼10 to 20 beads) were added to each well. The moment the self-indicating resin was added, it immediately changed to blue. To remove excess amine, methylisocyanate resin (100 mg, 0.15 mmol) was added, and the microplate was shaken until the blue color of the self-indicating resin in every well disappeared. The resin was filtered and washed with DCM. The combined filtrates were evaporated under reduced pressure to give 24 ureas as white solids or oils.

**Acknowledgment.** We thank the Combinatorial Centre of Excellence partners: GSK, AstraZeneca, Pfizer, Roche, Eli-Lilly, Organon, Nova-Biochem/Merck, Amersham/Nycomed Amersham, and Evotech OAI and the EPSRC (JIF initiative).

## References and Notes

(1) (a) Krchňák, V.; Holladay, M. W. *Chem. Rev.* **2002**, *102*, 61−91. (b) Blaney, P.; Grigg, R.; Sridharan, V. *Chem. Rev.* **2002**, *102*, 2607−2624. (c) Eames, J.; Watkinson, M. *Eur. J. Org. Chem.* **2001**, *7*, 1213−1224. (d) Drewry, D. H.; Coe, D. M.; Poon, S. *Med. Res. Rev.* **1999**, *19*, 97−148. (e) Ley,

S. V.; Baxendale, I. R.; Bream, R. N.; Jackson, P. S.; Leach, A. J.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. I.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, *23*, 3815−4195.

(2) (a) Dorman, L. C. *Tetrahedron Lett.* **1969**, *28*, 2319−2321. (b) Gisin, B. F. *Anal. Chim. Acta* **1972**, *58*, 248−249. (c) Reddy, M. P.; Voelker, P. J. *Int. J. Pept. Protein Res.* **1988**, *31*, 345−348. (d) Chen, C.; Ahlberg Randall, L. A.; Miller, R. B.; Jones, A. D.; Kurth, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 2661−2662. (e) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580−11581.

(3) (a) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598. (b) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147−157.

(4) (a) Salisbury, S. A.; Tremeer, E. J.; Davies, J. W.; Owen, E. D. I. A. *J. Chem. Soc., Chem. Commun.* **1990**, *7*, 538−540. (b) Campbell, D. A.; Bermak, J. C. *J. Am. Chem. Soc.* **1994**, *116*, 6039−6040. (c) Chu, S. S.; Reich, S. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1053−1058.

(5) Eberle, A. N.; Atherton, E.; Dryland, A.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1*, **1986**, 361−367.

(6) Krchňák, V.; Vágner, J.; Flegel, M.; Mach, O. *Tetrahedron Lett.* **1987**, *28*, 4469−4472.

(7) Krchňák, V.; Vágner, J.; Šafář, P.; Lebl, M. *Collect. Czech. Chem. Commun.* **1988**, *53*, 2542−2548.

(8) $^1$H NMR (400 MHz, 5% $CF_3CO_2D$ in $CD_3CN$): $\delta = 8.03$ (d, 2H, $J = 8.4$), 7.95 (d, 1H, $J = 6.8$), 7.82 (t, 1H, $J = 7.6$), 7.74 (t, 1H, $J = 7.8$), 7.58 (d, 1H, $J = 7.6$), 7.54 (d, 2H, $J = 8.4$), 7.47 (d, 1H, $J = 2.4$), 7.45 (s, 2H), 7.17 (d, 1H, $J = 2.0$); calcd mass for $C_{26}H_{15}Br_3O_7S$, 711.2; found, ESI-MS, *m*/*z*, negative $[M - H]^-$, 710.8.

(9) Ten percent of the original loading capacity of aminomethyl-NovaGel resin (0.74 mmol/g) was coupled to **1**, and the remaining amino groups on the resin were capped with acetic anhydride and TEA. The resin appeared negative in the ninhydrin test. Final loading of **1** was confirmed by elemental analysis: cald. Br/N = 1.71, found Br/N = 1.78 (average of seven samples).

(10) Leu for Leu-enkephalinamide and Val, Gln, Ile for acyl carrier protein (65−74) were more difficult to couple than the other residues.

(11) Hancock, W. S.; Prescott, D. J.; Vagelos, P. R.; Marshall, G. R. *J. Org. Chem.* **1973**, *38*, 774−781.

(12) Calcd mass for Leu-enkephalinamide, 554.3; found, ESI-MS, *m*/*z*, positive $[M + H]^+$, 555.2; calcd mass for acyl carrier protein (65−74) amide, 1061.6; found, ESI-MS, *m*/*z*, positive $[M + H]^+$, 1062.2.

(13) Booth, R. J.; Hodges, J. C. *J. Am. Chem. Soc.* **1997**, *119*, 4882−4886.

CC034010I