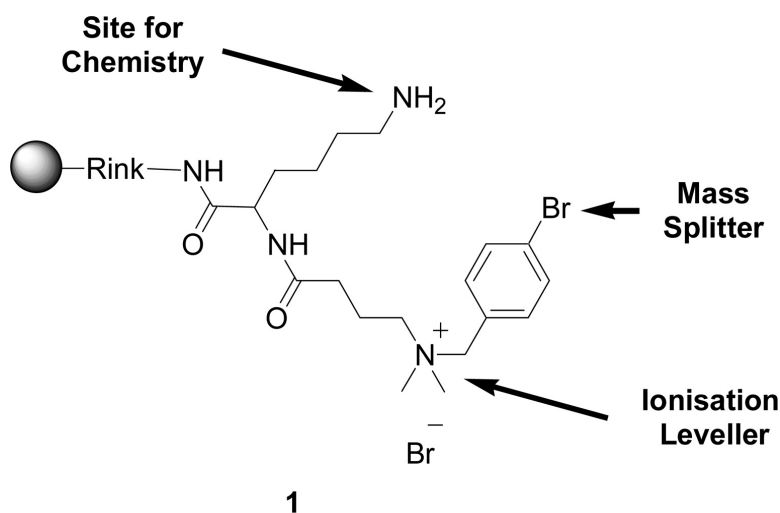Article

High Throughput Physical Organic Chemistry:
Analytical Constructs for Monomer Reactivity Profiling

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High Throughput Physical Organic Chemistry: Analytical Constructs for Monomer Reactivity Profiling

Christophe Portal,[†] Delphine Launay,[†] Andrew Merritt,[‡] and Mark Bradley*,[†]

Combinatorial Centre of Excellence, School of Chemistry, The University of Edinburgh, Edinburgh EH9 3JJ, UK, GSK Research and Development, Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY, UK

A polymer-supported analytical construct was used to quantify the reactivity of a range of monomers in the Ugi four-component condensation using positive electrospray ionization mass spectrometry (MS) as a quantitative analytical tool. The construct incorporated a bromo group to act as a peak splitter and a quaternary ammonium to act as a MS sensitizer and ionization leveler, thereby allowing direct quantitation of the cleaved adducts by MS. The relative reactivities of 10 carboxylic acids were quantified by the relative levels of product generated as determined by MS and 10 isonitriles, and 10 aldehydes were investigated in the same way. The effect of concentration variations on monomers reactivity and product profiles were rapidly determined using this approach, and the method opens up the way for studying, in a single pot, multiple reactions with a broad range of monomers under identical and self-consistent reaction conditions.

Introduction

Over the past few years, combinatorial synthesis has become a very fast and efficient technique for preparing a range of pharmacologically active compounds.[1,2] However, despite the ability to quickly provide a huge number of potential drug candidates, both split and mix or discrete compounds library syntheses are often not as efficient as desired to enable the production of a highly diverse set of pure compounds. Indeed, many combinations of reactants do not lead to the desired compound as the main product, which represents a major waste of time and resources. One of the main reasons for this reaction failure is that the vast variety of monomers that need to be chosen in order to ensure maximum diversity of the library also increases the differences in reactivity between the different building blocks, and even though optimization of reaction conditions are generally undertaken before full-scale library synthesis, this cannot cover all combinations of monomers implicated. Ideally, each monomer should be tested to ensure it is reactive enough to produce the desired product in good yield and purity to have it immediately ready for screening.

A method to evaluate the reactivity of monomers was recently reported by Parr et al. who described the use of a quantitative solid-phase analytical construct to evaluate the reactivity of amine monomers toward reductive amination.[3] The concept of analytical constructs, first introduced by Geysen,[4] was modified to include a ultraviolet (UV) chromophore used to allow quantitative deductions; however, this method requires both mass spectrometry (MS) and liquid chromatography/UV (LC/UV) analysis after cleavage from
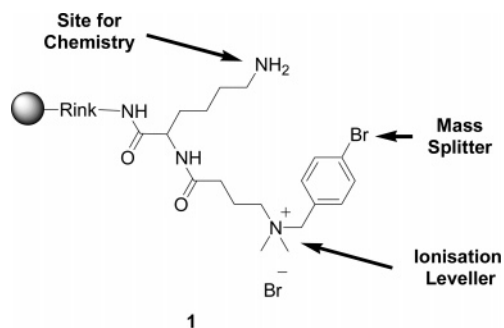
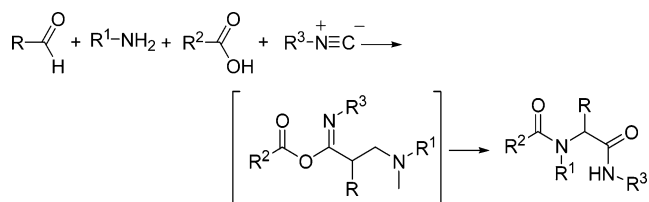**Figure 1.** Resin-bound analytical construct **1**.

Figure 2. The Ugi 4CC.

the resin to identify and quantify the products and link their concentration to monomer reactivities.

The methodology described herein allows the rapid profiling of the reactivity of a set of monomers by means of a single positive electrospray ionization MS (ESI+/MS) analysis. To do this, an analytical construct elaborated on the basis of the work of Carrasco was used.[5] A reactive functionality present on the construct was allowed to react with an array of monomers, and cleavage yielded a mixture of products, each attached to the common construct, on which ESI+/MS analysis could be performed. This analysis was made quantitative thanks to the presence on the construct of a quaternary ammonium species. As reported by Szewczyk,[6] such a group dominates ionization of the global structure

* E-mail: mark.bradley@ed.ac.uk.
[†] University of Edinburgh.
[‡] GSK Research and Development.

Scheme 1. Preparation of the Analytical Construct[a]
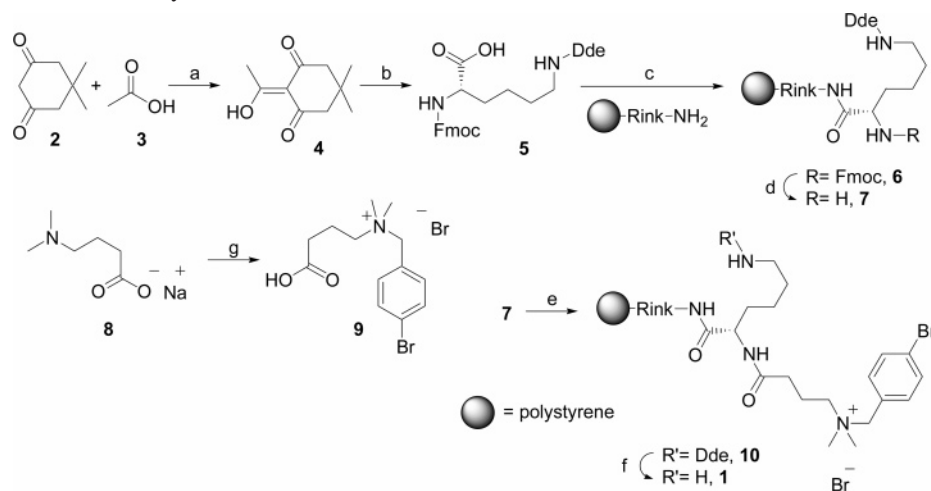
[a] Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, 36 h, 75%; (b) TFA (0.1 equiv), EtOH, reflux, 60 h, 54%; (c) Rink amine resin ($s = 0.85$ mmol/g, prepared from aminomethylated polystyrene (Polymer Laboratories, 1.11 mmol/g, 75–150 μm, 1–2% divinylbenzene), DIC, HOBT, CH_2Cl_2/DMF (7:3, v/v), 12 h; (d) 20% piperidine, DMF, 30 min; (e) **9**, DIC, HOBT, CH_2Cl_2/DMF (7:3, v/v), 12 h; (f) 80% $NH_2OH\cdot HCl$/imidazole, in *N*-methyl pyrrolidone/CH_2Cl_2 (1:1 v/v), 3 h; (g) (1-bromo-4-bromomethyl) benzene, CH_2Cl_2, 1 h, then Amberlite 200, CH_2Cl_2, 30 min, 98%.

and therefore links the intensity of the peak to the quantity of the corresponding compound in the mixture. The presence of an aryl bromide provided a known isotope pattern for the molecular ions allowing rapid identification of the products (see Figure 1).[7]

Since the intensities of the ESI+/MS peaks correlate to relative amounts of the various products, each value obtained can therefore be attributed to the reactivity of monomers. Thus, one ESI+/MS analysis gives a quantification of the reactivity of each monomer. The analytical construct used in this study had an amine functionality that was used as the amine entry for an Ugi four-component condensation (4CC). This multiple component reaction has the advantage of building quite complex α-acylamino amides from simple building blocks (Figure 2);[8] the technique has first been used to validate the methodology and then to evaluate the reactivity of 10 common aldehydes, 10 carboxylic acids, and 10 isonitriles in the Ugi 4CC.

Results and Discussion

Preparation of the Construct. The 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene (Dde) protecting group was prepared from dimedone **2** and acetic acid **3**[9] and condensed with Fmoc-Lys-OH to give Fmoc-Lys-(Dde)-OH **5**.[10] Coupling to polystyrene Rink amine resin and subsequent deprotection of Fmoc group afforded resin **7**. The analytical enhancer was prepared from sodium 4-dimethylaminobutyrate **8** which was alkylated with (1-bromo-4-bromomethyl) benzene to give ammonium salt **9**. Coupling to resin **7** gave resin **1**, after Dde deprotection (Scheme 1).[11]

Proof of the Method. To validate the methodology, it had to be proved that the MS method was quantitative and that ionization of the analytical construct, once cleaved, was dominated by the quaternary ammonium ion (i.e., independent of the product bound to it). To achieve this proof compounds **11**, **12**, **13**, and **14** were synthesized in parallel via an Ugi 4CC, using **1** as the resin bound amine entry (Chart 1).

Chart 1. Compounds Used to Validate the Method
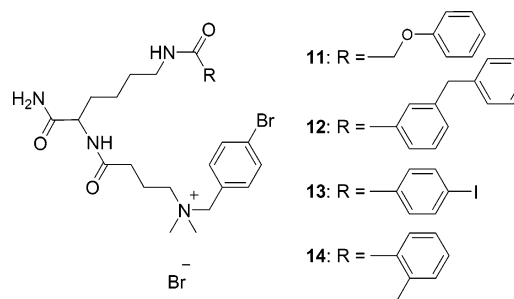
Following cleavage of the compounds from the solid support, each product was purified by semipreparative high-performance (HP)LC and analyzed through an online detection system comprising (i), a chemiluminescent nitrogen detector (CLND) (ii), an evaporative light scattering detector (ELSD) (iii), a diode array detector (DAD) (iv), and an electrospray ionization mass spectrometer (ESI/MS). Quantitation was then determined according to the peak areas for CLND[12] and precalibrated ELSD,[13] while MS quantification was achieved by calculation of the abundance of the compound by using the MS trace and the intensity of the concerned peak.

The spectrum obtained by ESI+/MS analysis demonstrated all the properties expected; thus each compound was well ionized and detectable, and in each case, only the molecular peak was present and gave the expected $^{79}Br/^{81}Br$ patterns, which allowed them to be rapidly differentiated from any background noise (Figure 3).

The intensities of the ESI+/MS peaks obtained for the analyses of different concentrations of solutions of **11**, **12**, **13**, and **14** were plotted vs their respective ELSD areas and CLND areas (Chart 2). The linearity between the various methods analyses was very good (regression coefficients were 0.997 for ELSD vs MS and 0.994 for MS vs concentrations as determined by CLND).

Additionally, the method was validated by checking the effect of other potentially ionisable groups bound to the construct. Two products were prepared in a single-pot Ugi
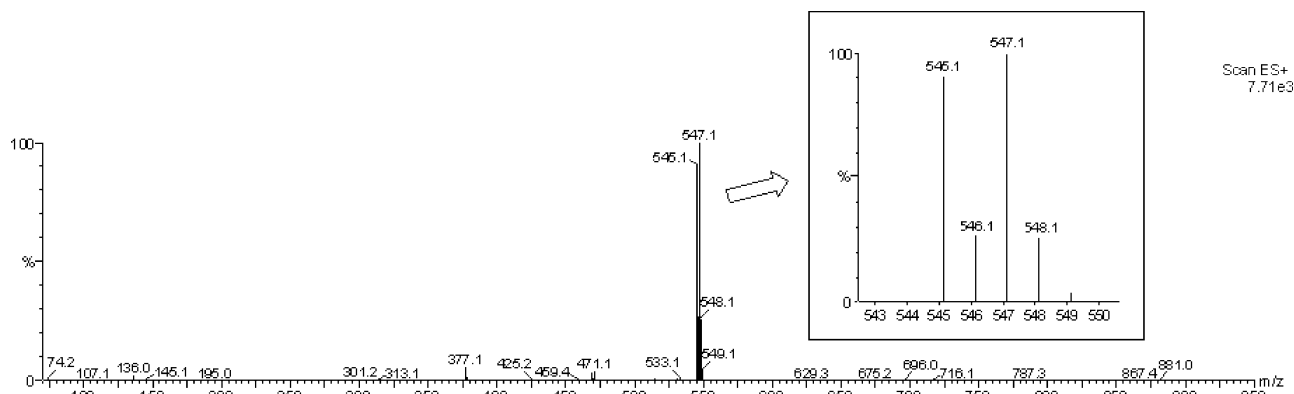
Figure 3. MS spectrum obtained for compound **14** and an expansion showing the bromine isotope pattern.

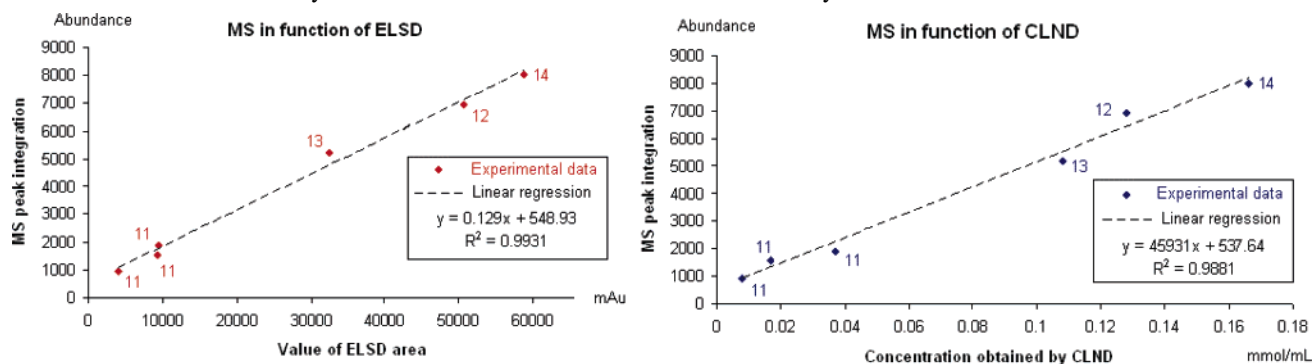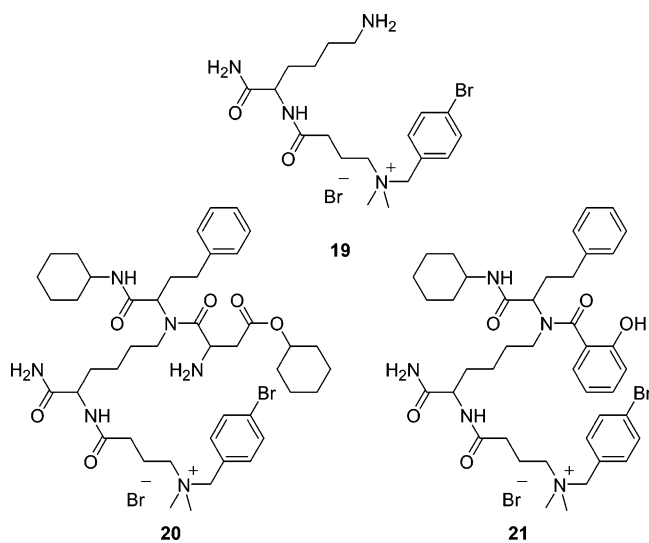**Chart 2.** Proof of the Linearity between ESI+/MS and ELSD and CLND Analyses

Chart 3. Mixture of Products Analyzed by ESI+/MS and ELSD

Table 1. Composition of the mixture of **19**, **20** and **21** determined by ESI+/MS and ELSD

compound	composition of the mixture	
	ESI+/MS	ELSD
19	45%	54%
20	10%	6%
21	45%	40%

Chart 4. The Ten Carboxylic Acids Used in Ugi 4CC

4CC, using **1** as the resin bound amine entry, hydrocinnam-aldehyde **15**, cyclohexyl isonitrile **16**, Boc-Asp(OChex)-OH **17**, and salicylic acid **18**, to give a mixture of unreacted amine **19** and products **20** and **21** (Chart 3).

The composition of the mixture was analyzed by ELSD and ESI+/MS. As shown in Table 1, the results obtained demonstrate the ability of the method to quantify products in a mixture, independently to what is attached to the construct.
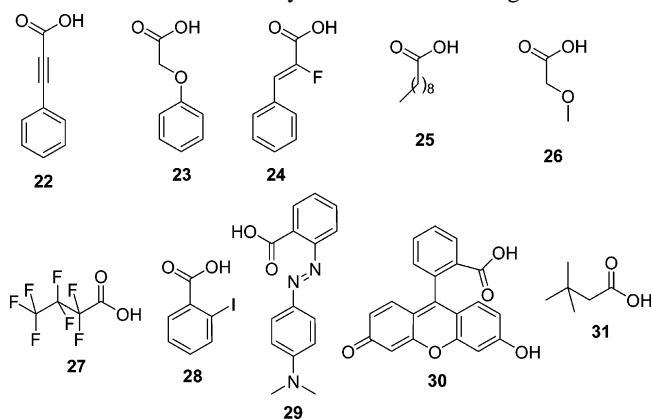
Since both studies confirmed that the method was quantitative, further investigations could be undertaken.

Carboxylic Acid Reactivity Profiling. Because the method was quantitative, it was possible to use it to determine the composition of a mixture of different compounds bound to the analytical construct. Thus by reacting the solid supported amine analytical construct with an isonitrile, an aldehyde, and 10 different carboxylic acids (as shown in Chart 4), 10 Ugi 4CCs would occur and a mixture of products result.

The reactivity of each acid in the assay could then be measured after cleavage by means of a single ESI+/MS analysis (the other species chosen for the reaction were

Chart 5. Values and Standard Deviations Obtained for the Carboxylic Acid Reactivity Profiling

Carboxylic acid reactivity profiling

Assay 1: 0.25 equiv.
Average conversion: 74%

Assay 2: 0.50 equiv.
Average conversion: 69%

Assay 3: 0.75 equiv.
Average conversion: 73%
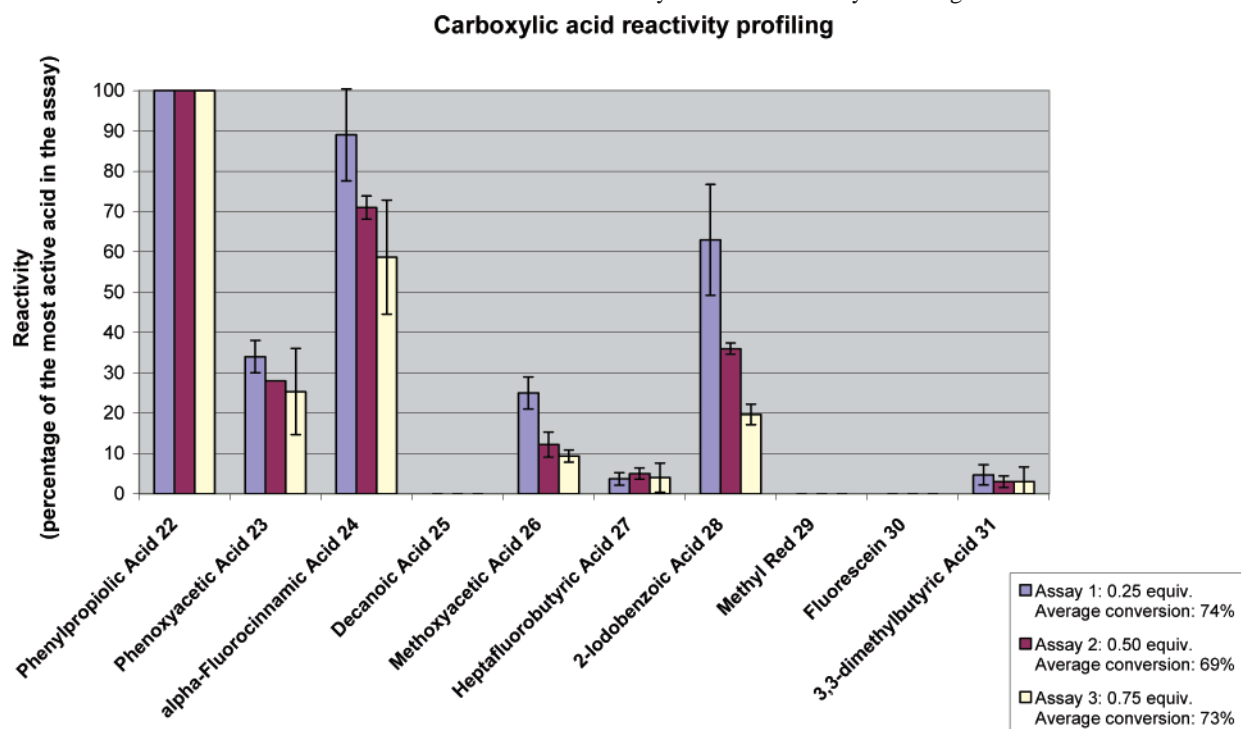
hydrocinnamaldehyde **15** and cyclohexyl isonitrile **16**, both components having good reactivity in the Ugi 4CC. Five equivalents (61 mM) were used relative to the amine entry in order to guarantee it would not be the limiting factor in the reaction (pseudo first order). The reactions were performed in methanol/CH$_2$Cl$_2$ (1:1, v/v) under microwave irradiation for 30 min at 120 °C.[14] Several concentrations of acid were used in order to establish if the reactivity of one of the acids would dominate the others', thus 0.25 equiv (3.0 mM), 0.5 equiv (6.1 mM), and 0.75 equiv (9.1 mM) of each acid were used respectively for assays 1, 2, and 3. After each reaction, the products were cleaved from the Rink linker using a solution of trifluoroacetic acid (TFA) in CH$_2$Cl$_2$ (20% v/v).

The ESI+/MS data obtained for each assay was treated with the Masslynx software, which allowed the peaks that presented a bromine pattern to be extracted from the background noise. After measurement of the intensity of each peak, a value was attributed to each acid relating to its reactivity (three assays were performed for each value of the monomer concentration). Thus, the reactivity of each monomer as a percentage of the most active in the assay is reported in Chart 5 as well as the standard deviation. By use of the intensity of the MS peak relative to the starting amine, the conversion was also calculated for each assay.

Clearly, phenylpropiolic acid **22** had the highest reactivity, followed by α-fluorocinnamic acid **24** and 2-iodobenzoic acid **28**. Bulky structures such as methyl red **29** and fluorescein **30** were revealed to be essentially unreactive, as well as decanoic acid **25**, which was quite surprising. At high concentrations the reactivity of phenylpropiolic acid **22** in some cases dominated the chemistry even though the relative order of reactivity was unchanged, with, for example, the values measured for α-fluorocinnamic acid **24** and 2-iodobenzoic acid **28** being lowered in case of 0.75
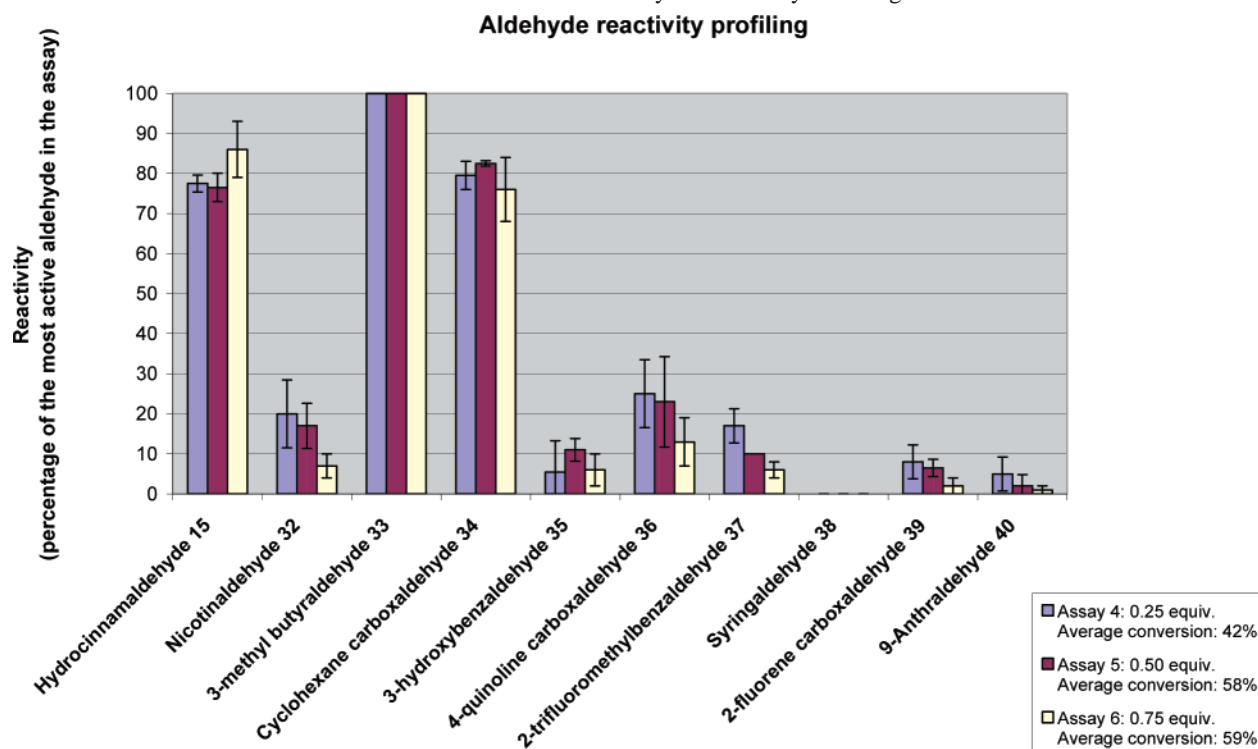
Chart 6. The Ten Aldehydes Used in Ugi 4CCs
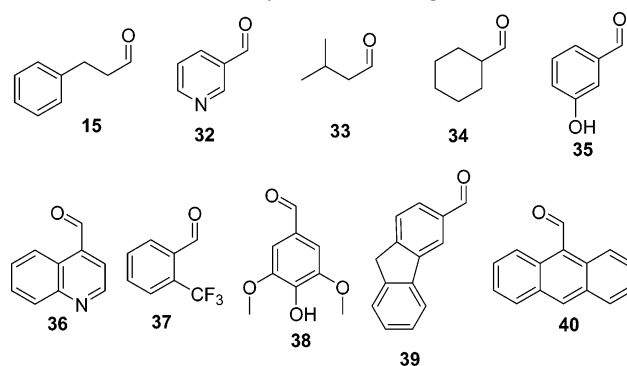
equivalent of each acid. To be able to give a representative value of the reactivity of each acid, it was therefore necessary to maintain the amount of each acid low enough to avoid saturating the experiment and losing the competitive effect.

It also came out of this study that each acid had a completely different behavior with regards to the reaction. A library synthesis that would involve the 10 acids picked for this study would be inefficient as more than 50% of the reaction mixtures would result in no product or dramatically poor yields; thus with this technique, it becomes possible to exclude unreactive monomers from library synthesis.

Aldehyde Reactivity Profiling. The same study was carried out on 10 aldehydes (Chart 6). Five equivalents of phenylpropiolic acid **22** and five equivalents of cyclohexyl isonitrile **16** were used for each assay (61 mM), and again, the amount of aldehyde was varied. Thus 0.25 equiv (3.0 mM), 0.5 equiv (6.1 mM), and 0.75 equiv (9.1 mM) of each aldehyde were used for assays 4, 5, and 6, respectively.

The average conversions for assays 4, 5, and 6 were lower than the ones observed in case of the acids. A similar process as described above allowed the reactivity of each aldehyde

Chart 7. Values and Standard Deviations Obtained for the Aldehyde Reactivity Profiling

Chart 8. The Ten Isonitriles Used in Ugi 4CCs
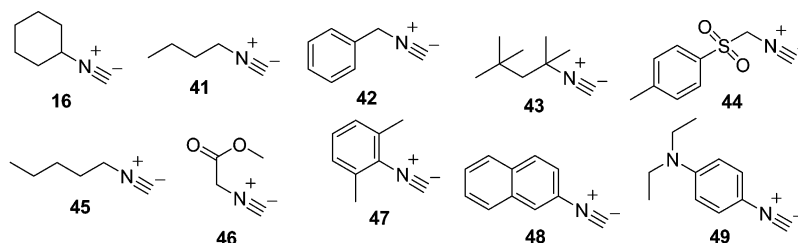
to be determined from the ESI+/MS spectra. The same conclusions as those observed by Tempest[15] and Kim[16] in more traditional experiments were drawn: aliphatic aldehydes as 3-methyl butyraldehyde **33** and cyclohexane carboxaldehyde **34** had very good reactivities (Chart 7). Hydrocinnamaldehyde **15** also showed good activity. Nicotinaldehyde **32**, 4-quinoline carboxaldehyde **36**, and 2-trifluoromethylbenzaldehyde **37** had only a reactivity of around 10 percent of the best monomer, while syringaldehyde **38** did not react in any of the three assays. None of the aldehydes dominated the reactivity as determined by the flat effect of increasing the quantity of monomer in the reaction.

Isonitrile Reactivity Profiling. The same procedure was applied for the reaction of the 10 isonitrile monomers, listed in Chart 8.
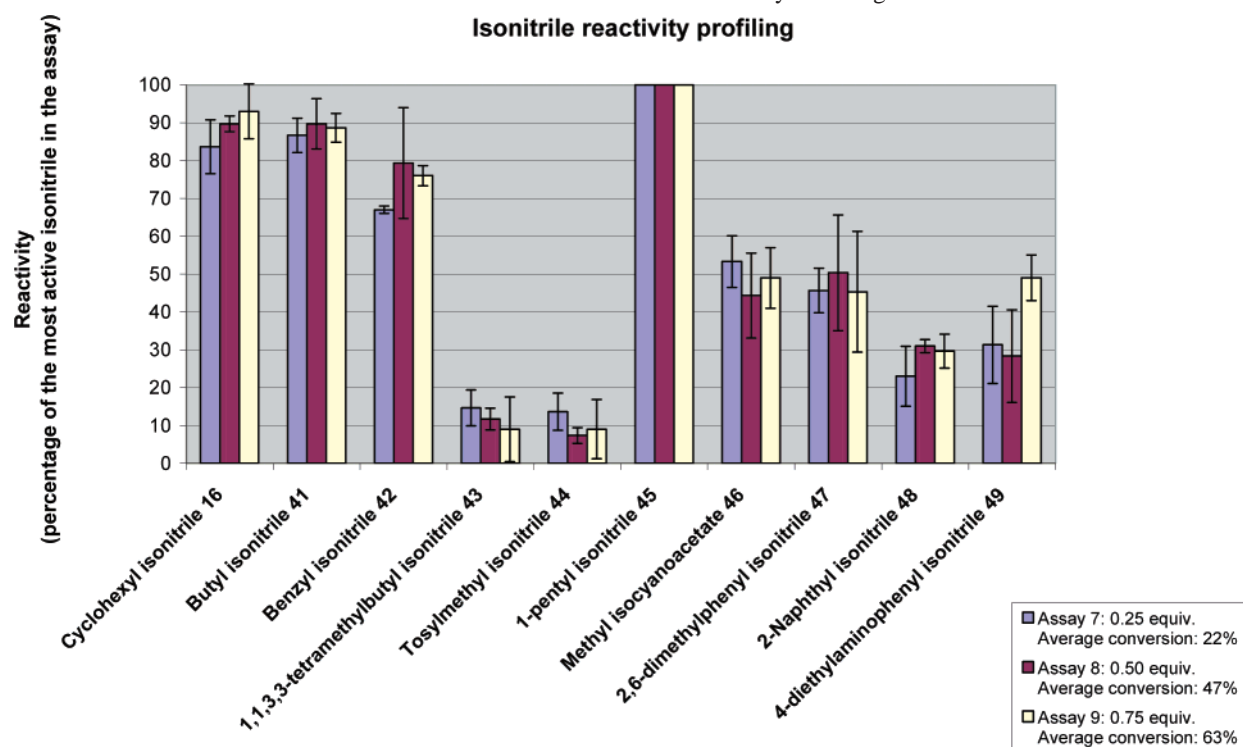
For these assays, phenylpropiolic acid **22** and hydrocinnamaldehyde **15** were used, respectively, as the acid and the aldehyde entries of the Ugi 4CC (5 equiv of each, 61 mM). Butyl isonitrile **41**, cyclohexyl isonitrile **16**, and benzyl isonitrile **42** had good reactivity compared to the reference, which was 1-pentyl isonitrile **45** (Chart 9). The amount of isonitrile used for assays 7, 8, and 9, which was 0.25 equiv (3.0 mM), 0.5 equiv (6.1 mM), and 0.75 equiv (9.1 mM) did not affect the reactivity. However it came that the conversion of the starting amine was very dependent on the

quantity of isonitrile involved in the reaction as showed on Chart 9. 1,1,3,3-Tetramethylbutyl isonitrile **43** and tosylmethyl isonitrile **44** showed poor reactivity toward the Ugi 4CC and are not advisable for combinatorial synthesis under the described conditions.

Conclusion

In conclusion, a high throughput tool to quantify the reactivity of combinatorial chemistry monomers has been developed. An analytical construct was built up and has proven to be very reliable to quantify products in a mixture in a HT manner. This property allowed the rapid quantification of the reactivity of carboxylic acids, aldehydes, and isonitriles monomers during a series of Ugi 4CCs; the quantitative study of the monomers reported here only took a few hours per family (three concentrations tested), allowing fast discrimination of unreactive compounds, so that the synthetic process can be undertaken with a panel of building blocks having the same level of reactivity. The values of the reactivities obtained were comparable to those already reported in the literature,[15,16] and the use of this construct can extended to rapidly evaluate the reactivity of monomers in a broad spread of reactions making it useful in undertaking general monomer reactivity profiling as well as high throughput physical organic chemistry.

Chart 9. Values and Standard Deviations Obtained for the Isonitrile Reactivity Profiling

Isonitrile reactivity profiling

Reactivity (percentage of the most active isonitrile in the assay)

Cyclohexyl isonitrile 16, Butyl isonitrile 41, Benzyl isonitrile 42, 1,1,3,3-tetramethylbutyl isonitrile 43, Tosylmethyl isonitrile 44, 1-pentyl isonitrile 45, Methyl isocyanoacetate 46, 2,6-dimethylphenyl isonitrile 47, 2-Naphthyl isonitrile 48, 4-diethylaminophenyl isonitrile 49

Assay 7: 0.25 equiv. Average conversion: 22%
Assay 8: 0.50 equiv. Average conversion: 47%
Assay 9: 0.75 equiv. Average conversion: 63%

Experimental

Instrumentation. NMR spectra were recorded on a Bruker AC-300 spectrometer in the solvents indicated at 298 K. Chemical shifts are reported on the δ scale in ppm and were referenced to residual solvents resonances. IR spectra were obtained with neat compounds on a Fourier transform infrared (FTIR) Perkin-Elmer 2000 Spectrometer (Beaconsfield, Bucks, England) coupled with an AutoIMAGE FTIR microspectrometer (Beaconsfield, Bucks, England), 32 scans, resolution $\pm$ 8 cm^{-1}. HPLC/ELSD analyses were obtained using an Agilent 1100 series system (eluent A, water + 0.1% formic acid; eluent B, methanol + 0.1% formic acid; gradient, 95–5% A over 10 min then 5–95% A over 3 min) coupled to a Polymer Lab 100 ES ELS Detector. Eluents used were analytical grade. ESI+/MS analyses were carried out on an Agilent Technologies LC/MSD Series 1100 quadrupole mass spectrometer (QMS) using electrospray positive ionization. Reactions under microwave irradiation were performed in a SmithSynthesizer from Biotage. The version of the Masslynx software that was used is version 2.2 build 9.

Preparation of Dde–OH (4).[9] Dimedone (**2**) (11.5 g, 82 mmol) was dissolved in DMF (175 mL) with acetic acid (**3**) (4.95 g, 1 equiv), DCC (17 g, 1 equiv), and (dimethylamino)-pyridine (DMAP) (10 g, 1 equiv). The reaction was finished over 36 h. Precipitating dicyclohexylurea (DCU) was removed by filtration, and the solvent was evaporated in vacuo. After dissolution in ethyl acetate, the organic phase was dried with MgSO$_4$ and the solvent evaporated to afford Dde–OH as an orange oil (11.9 g, 75%). IR ν (cm^{-1}): 3270, 2927, 1719, 1679, 1504–1450, 1208, 785–699. ^{1}H NMR (300 MHz, CDCl$_3$, δ, ppm): 13.98 (1H, s), 2.59 (3H, s), 2.52 (2H, s), 2.34 (2H, s), 1.06 (6H, s). ^{13}C NMR + DEPT 135

(75 MHz, CDCl$_3$, δ, ppm): 202.4, 197.9, 195.2, 112.3, 52.5, 46.9, 30.8, 28.5, 28.2.

Preparation of N_α-Fmoc-N_ϵ-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)ethyl]-lysine (Fmoc-Lys(Dde)-OH (5).[10] Trifluoroacetic acid (84 μL, 1.1 mmol) was added to a stirred suspension of Fmoc-Lys-OH (4.04 g, 10.7 mmol) and Dde–OH (**4**) (4 g, 2 equiv) in ethanol (90 mL) at room temperature. The mixture was then refluxed for 60 h (reaction monitored with analytical thin-layer chromatography (ethyl acetate/hexane 95:5 v/v, R_f = 0.21). The solvent was evaporated and the orange residue dissolved in ethyl acetate (150 mL). The organic solution was washed with 1 M aqueous KHSO$_4$ (2 × 175 mL). After drying and concentrating in vacuo, the yellow oil was triturated three times with hexane to remove unreacted Dde–OH to give **5** as a white crystalline solid (3.1 g, 54%). ESI+/MS: m/z = 533.3 (M + H)$^+$. ^{1}H NMR (300 MHz, CDCl$_3$, δ, ppm): 13.32 (1H, s), 7.74 (2H, d, J^3 = 7.3 Hz), 7.58 (2H, d, J^3 = 6.8 Hz), 7.37 (2H, t, J^3 = 7.1 Hz), 7.27 (2H, t, J^3 = 7.3 Hz), 5.79 (1H, d, J^3 = 7.9 Hz), 4.37 (1H, m), 4.19 (2H, m), 3.38 (1H, m), 2.55 (2H, s), 2.36 (3H, s), 1.97 (2H, m), 1.96–1.53 (6H, m), 1.00 (6H, s). ^{13}C NMR + DEPT 135 (75 MHz, CDCl$_3$, δ, ppm): 198.2, 174.5, 174.0, 156.2, 143.9, 143.8, 141.3, 127.7, 127.0, 125.1, 120.0, 107.9, 67.1, 53.4, 52.4, 43.3, 32.0, 31.6, 30.1, 28.4, 28.2, 22.6, 22.4, 18.1, 14.1.

Preparation of Rink Amine Polystyrene Resin. The Fmoc-Rink Linker (970 mg, 1.8 mmol) was dissolved in CH$_2$Cl$_2$ (7 mL) and DMF (3 mL). Hydroxybenzotriazole was added (243 mg, 1.5 equiv), followed after 10 min of stirring by DIC (279 μL, 1.5 equiv). After 20 min of stirring, aminomethylated polystyrene resin (1.08 g, s = 1.11 mmol/g) in CH$_2$Cl$_2$/DMF (15 mL, 7:3 v/v) was added. The reaction was stirred over 15 h until a ninhydrin test was negative. The resin was washed with DMF (15 mL, 3 times), CH$_2$Cl$_2$,

(15 mL, 3 times), methanol (15 mL, 3 times), and diethyl ether (15 mL, 3 times). Fmoc group deprotection was performed by treating the resin with Piperidine/DMF solution (20% v/v) for 30 min. The resin was washed with DMF (15 mL, 3 times), CH_2Cl_2, (15 mL, 3 times), methanol (15 mL, 3 times), and diethyl ether (15 mL, 3 times). The resin was dried in vacuo overnight to afford white colored Rink amine resin (1.5 g). IR ν (cm^{-1}): 3250, 2922, 1681, 1503−1452, 1208, 785−699.

Preparation of Resin 7. Fmoc−Lys(Dde)−OH **5** (2 g, 3.7 mmol) was dissolved in CH_2Cl_2/DMF (40 mL, 7:3 v/v). Hydroxybenzotriazole was added (499 mg, 2 equiv), followed after 10 min of stirring by DIC (576 μL, 2 equiv). After 20 min of stirring was added Rink amine resin (3.3 g, s = 0.85 mmol/g) in CH_2Cl_2/DMF (7:3 v/v, 50 mL). The reaction was stirred over 15 h until a ninhydrin test was negative. The resin was washed with DMF (40 mL, 3 times), CH_2Cl_2, (40 mL, 3 times), methanol (40 mL, 3 times), and diethyl ether (40 mL, 3 times). Resin **6** was dried in vacuo overnight to afford a buff-colored resin (4.2 g). Fmoc group deprotection was performed as previously described; the resin was then dried in vacuo overnight to afford the title resin **7**.

Preparation of (4-Bromo-benzyl)-(4-carboxy-butyl)-dimethylammonium Bromide (9). NaOH (4 g, 100 mmol) in ethanol (15 mL) was added to a stirred solution of *N,N*-dimethylaminobutyric acid (8.35 g, 50 mmol) in CH_2Cl_2 (100 mL). Solvent was evaporated, and the acid was dissolved in CH_2Cl_2. 4-Bromoethylbenzyl bromide (13.75 g, 1.1 equiv) was added dropwise during 30 min. The mixture was stirred over 30 min at room temperature. The salt was filtered and washed twice with CH_2Cl_2 (20 mL) and then stirred in CH_2Cl_2 on a linear shaker in the presence of the activated Amberlite 200 to give a white salt. (98%). ESI+/MS: m/z = 300.0 (M$^+$). ESI+/HRMS: $C_{13}H_{19}NBr_2O_2$ calculated m/z = 300.0594 (M$^+$) measured m/z = 300.0593. IR ν (cm^{-1}): 3024, 2967, 1725, 1573, 501. ^{1}H NMR (300 MHz, d^6-DMSO, δ, ppm): 7.76 (2H, d, J^3 = 8.4 Hz), 7.66 (2H, d, J^3 = 8.4 Hz), 4.69 (2H, s), 3.35 (2H, m), 3.03 (6H, s), 2.06 (2H, m), 1.98 (2H, m). ^{13}C NMR + DEPT 135 (75 MHz, d^6-DMSO, δ, ppm): 175.9, 136.1, 132.7, 128.6, 124.9, 65.6, 65.5, 50.0, 34.3, 20.0.

Preparation of Resin 1. Acid **9** (1 g, 2.8 mmol) was dissolved in CH_2Cl_2 (14 mL) and DMF (6 mL). HOBt was added (383 mg, 2 equiv) and after 10 min of stirring, DIC (441 μL, 2 equiv) was added. After 20 min of stirring, resin **7** (2 g, s = 0.66 mmol/g) in CH_2Cl_2/DMF (7:3 v/v, 30 mL) was added. The reaction was stirred over 15 h until a ninhydrin test was negative. The resin was washed with DMF (30 mL, 3 times), CH_2Cl_2, (30 mL, 3 times), methanol (30 mL, 3 times), and diethyl ether (30 mL, 3 times). The resin was dried in vacuo overnight to afford resin **10** as a buff colored resin (3 g). Polystyrene bound analytical construct **1** was afforded through deprotection of resin **10** by swelling it in 80% $NH_2OH \cdot HCl$/imidazole in *N*-methyl pyrrolidone/CH_2Cl_2 (1:1 v/v) for 3 h.[11]

Microwave-Assisted Solid-Supported Ugi 4CCs: Example of Carboxylic Acid Reactivity Study. Resin (75 mg, **1**, s = 0.61 mmol/g) was swollen with CH_2Cl_2/MeOH (50% v/v, 1 mL) in a 5-mL microwave vial. To the resin was added the mixture of 10 carboxylic acids in CH_2Cl_2/MeOH (50% v/v, 0.5 mL) and then hydrocinnamaldehyde (**15**) (41 mg, 5 equiv) in solution in CH_2Cl_2/MeOH (50% v/v, 0.5 mL). The vial was sealed and placed on a linear shaker for 30 min before cyclohexyl isonitrile (**16**) was added (47 μL, 5 equiv). The mixture was then microwave irradiated for 30 min at 120 °C. The resin was washed with DMF (1 mL, 5 times), CH_2Cl_2, (1 mL, 5 times), methanol (1 mL, 5 times), and diethyl ether (1 mL, 5 times). The products were then cleaved form the resin with 1 mL of trifluoroacetic acid/CH_2Cl_2 (20% v/v, 1 mL, 15 min). Toluene was added to the mixture before solvents were removed in vacuo to prevent products from being in the presence of concentrated trifluoroacetic acid.

References and Notes

(1) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2001**, *5*, 273−284.
(2) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2003**, *7*, 308−325.
(3) Parr, N. J.; McKeown, S. C.; Lindvall, M. K.; Lorthioir, O. E.; Congreve, M. S.; Watson, S. P. *Lett. Org. Chem.* **2004**, *1*, 87−92.
(4) Geysen, H. M.; Wagner, C. D.; Bodnar, W. M.; Markworth, C. J.; Parke, G. J.; Schoenen, F. J.; Wagner, D. S.; Kinder, D. S. *Chem. Biol.* **1996**, *3*, 679−688.
(5) Carrasco, M. R.; Fitzgerald, M. C.; Oda, Y.; Kent, S. B. H. *Tetrahedron Lett.* **1997**, *38*, 6331−6334.
(6) Szewczyk, J. W.; Zuckerman, R. L.; Bergman, R. G.; Ellman, J. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 216−219.
(7) Lane, S. J.; Pipe, A. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 782−793.
(8) For review, see: Gokel, G.; Lüdke, G.; Ugi, I. In *Isonitrile Chemistry*; Ugi, I., Ed.; Academic Press: New York, 1971; p 145.
(9) Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1998**, *39*, 1603−1606.
(10) Chhabra, S. R.; Parekh, H.; Khan, A. N.; Bycroft, B. W.; Kellam, B. *Tetrahedron Lett.* **2001**, *42*, 2189−2192.
(11) Diaz-Mochon, J. J.; Bialy, L.; Bradley, M. *Org. Lett.* **2004**, *6*, 1127−1129.
(12) Fitch, W. L.; Szardenings, A. K.; Fujinari, E. M. *Tetrahedron Lett.* **1997**, *38*, 1689−1692.
(13) Fang, L. L.; Wan, M.; Pennacchio, M.; Pan, J. M. *J. Comb. Chem.* **2000**, *2*, 254−257.
(14) Hoel, A. M. L.; Nielsen, J. *Tetrahedron Lett.* **1999**, *40*, 3941−3944.
(15) Tempest, P. A.; Brown, S. D.; Armstrong, R. W. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 640−642.
(16) Kim, S. W.; Bauer, S. M.; Armstrong, R. W. *Tetrahedron Lett.* **1998**, *39*, 6993.