Pergamon

Tetrahedron Letters, Vol. 38, No. 29, pp. 5099-5102, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01136-2

Rapid In-Plate Generation of Benzimidazole Libraries and Amide Formation Using EEDQ

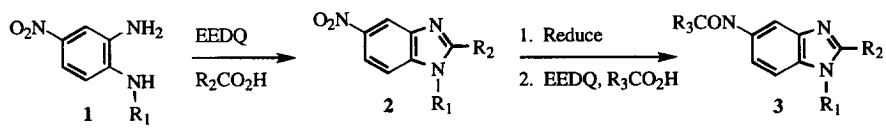
James B. Thomas, Michael J. Fall, Julie B. Cooper, Jason P. Burgess, and F. Ivy Carroll*

Chemistry and Life Sciences, Research Triangle Institute, P O Box 12194, Research Triangle Park, North Carolina 27709, USA

Abstract: A solution phase method for the preparation of etonitazene-related benzimidazoles and a general method for the preparation of amide derivatives in 96-well format have been developed for the generation of libraries of compounds in parallel. © 1997 Elsevier Science Ltd.

The use of combinatorial chemistry as a tool for drug discovery has seen tremendous advances in recent years as is evident from the numerous publications devoted to the topic.[1] To accelerate the drug development process, the bulk of this effort has been directed towards harnessing solid-phase synthesis for use in the generation of small-molecule libraries, including benzimidazole libraries.[2] In our efforts related to the discovery of novel binding sites within several classes of seven transmembrane receptors, we have explored methods for the rapid formation of both amides and benzimidazoles for initial screening studies. Since the libraries that we planned were relatively small sets of related compounds and since we wanted to prepare these sets in parallel, the solution-phase approach was chosen.

Scheme 1

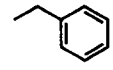
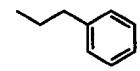
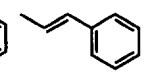
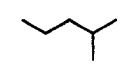
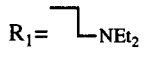


Earlier reports from this laboratory[3] demonstrated that the coupling reagent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)[4] could be used to prepare benzimidazole compounds related to the analgesic etonitazene, **2** (R_1=$CH_2CH_2NEt_2$, R_2=CH_2p-$EtOC_6H_4$), from readily available nitroanilines[5] exemplified by **1** (R_1=$CH_2CH_2NEt_2$). As this reaction sequence produced relatively pure material directly from the reactions, it seemed likely that it could be adapted to rapid parallel synthesis directly in 96-well plates. The principle obstacle to be overcome was that the reaction required several days to complete, and several additions of the coupling reagent were needed (2.5–3 equivalents). Since it would be desirable to use unpurified products in the screening without resorting to work-ups, limiting additions of reagents that would later be considered impurities would be advantageous. We discovered that the previously reported process could be modified such that etonitazene could be prepared without solvent simply by combining all of the ingredients together in their

solid form followed by heating to 120 °C under aspirator vacuum. For example, using only 1.5 equivalents of EEDQ and carboxylic acid on scales ranging from 5 μg to 10 g provided the required benzimidazoles in good yield, and work-up was not required as the by-products, ethanol and quinoline, were removed under the reaction conditions. The impurities detected were derived from the 0.5 equivalents excess of carboxylic acid and EEDQ used to drive the reaction to completion. This was an important discovery as it demonstrated that the benzimidazoles could be formed in minutes relative to the days required in the previous process. For the library generation, dimethylformamide (DMF) was used as it readily dissolved all of the reagents and starting materials and was removed under the reaction conditions facilitating distribution of the reactants using multi-channel pipettors. Using the process illustrated in Scheme 1, numerous analogs of etonitazene required for our studies were quickly prepared in yields and purities[6] comparable with R_2=CH_2Ph, R_1=$CH_2CH_2NEt_2$ in Table 1.

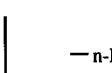
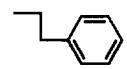
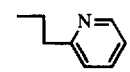
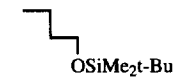
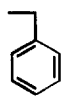
More diverse libraries possessing general structure **3** were generated using essentially the same process described above. While numerous excellent methods exist for the formation of amide bonds in solution[7] and on solid phase,[8] the success of EEDQ in the benzimidazole synthesis prompted us to study the reagent for use in a direct in-plate EEDQ-mediated amide formation process as we felt it would find general application since it uses air-stable, readily available carboxylic acids as the starting material and does not require work-up. In the development of this method, we evaluated several different variations at each of the three positions R_1–R_3, as well as determining the generality of the method on several other types of amines, Tables 1, 2, and 3.

Table 1. Representative Benzimidazoles (**2**) with Variation in Position 2.

R_1= \ R_2=						
	76 (81)[a]	79 (81)	88 (84)	89 (91)	65 (62)	68 (70)

[a] Yield (Purity)

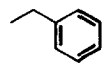
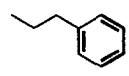
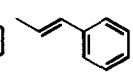
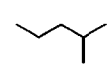
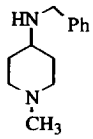
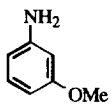
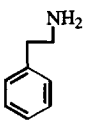
Table 2. Representative Benzimidazoles (**2**) with Variation in Position 1.

R_1= \ R_2=				
	83 (86)	NR	80 (87)	69 (81)

In general, all of the carboxylic acids evaluated produced the desired benzimidazoles, but none worked as well as phenyl alkyl acids. Table 2 illustrates that many variations in the position R_1 are acceptable, but as the case of R_1= CH_2CH_2Ph shows, not all variations are permitted. The use of a protecting group was necessary to prevent ester formation during cyclization when the R_1 possessed a hydroxyl group. While EEDQ is known to tolerate phenols and alcohols during reaction, we have found that the yields and purities are depressed somewhat due to acylation. The difference being the high temperatures used relative to standard peptide coupling conditions. Nevertheless, the amine competes favorably for the mixed anhydride intermediate formed from the carboxylic acid and EEDQ *in situ*. A general trend in purity appears to be that as the nucleophilicity

of the amine increases, so does the amount of a process impurity which is the ethyl carbamate of the particular amine used. Interestingly, the use of N-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ)[9] increases this impurity at the elevated temperatures used in this process.

Table 3.

RCO_2H, R=						
2A[a]	[b]53 (54)	70 (74)	87 (92)	85 (97)	69 (74)	82 (97)
	74 (62)	63 (55)	93 (94)	97 (98)	62 (57)	91 (96)
	75 (75)	58 (59)	97 (90)	85 (80)	80 (71)	83 (86)
	48 (52)	85 (88)	87 (90)	83 (82)	74 (76)	67 (77)

[a] **2A** is the reduced form of compound **2** in Scheme 1. [b] Yield (Purity)

As shown in Scheme 1, the last step of this process requires reduction of the nitro group of the benzimidazole, **2**, to aniline prior to amidation. While this has been acheived in many different ways,[10] we found that this procedure can be carried out in-plate using Raney nickel and hydrazine followed by filtration though celite in a Polyfiltronics™ 96-well filtration system; however, we prefer to perform these in vials followed by an aqueous work-up. Individual reduced benzimidazoles can then be amidated or converted into other desired analogs. This process provides a purer product and avoids unnecessary contamination. However, the principle reason for not performing the reaction in-plate is that, as is the case in parallel processing, time is required to utilize all of the wells from the benzimidazole plate and the reduced benzimidazoles darken with age. While the in-plate reduction would be fine as a finishing step, performing the reaction in vials provides fresh material for processing in the amidation reaction. Yields for the reduction step range from 85–95%.

Using this approach for library synthesis, thousands of compounds were prepared for evaluation in a relatively short period of time. We are currently preparing derivatives of general structure **3** and reports on this work will appear in due course.

General Procedure for Benzimidazole Production. To 1 equivalent of nitroaniline dissolved in DMF (1M) was added 1.5 equivalents of both EEDQ and acid derivative both in DMF. All pipetting was conducted in a fume hood in the atmosphere. The mixture was then heated to 100 °C for 1.5 h in a vacuum oven at atmospheric pressure, then the pressure was lowered to 30 in Hg and the temperature raised to 170 °C and

held for 4–5 h. After this time, the samples were cooled and diluted for screening. **CAUTION**: It is advised that the oven be allowed to cool under vacuum prior to opening to remove odors.

General Procedure for Nitrobenzimidazole Reduction. To a stirring solution of nitrobenzimidazole (0.055M) in ethanol was added moist Raney nickel (1 mg/0.002 mmol benzimidazole) and 10 equivalents hydrazine hydrate. This was stirred until no starting material remained as indicated by TLC. After filtration through celite, the ethanol was removed and the residue dissolved in $CHCl_3$, washed twice with water, and dried over sodium sulfate.

General Procedure for Amide Production. To 1 equivalent of amine dissolved in DMF (1M) was added 1.0 equivalents of both EEDQ and acid derivative both in DMF. The temperature was raised to 100 °C, and the pressure was lowered to 30 in Hg. Heating was maintained for 3 h or until all of the DMF and quinoline had been removed. More unreactive amines may require an initial heating period.

Acknowledgment. The authors wish to thank Dr. Steven Kaldor, Lilly Research Laboratories, Indianapolis, IN 46285-0540, for his helpful suggestions in the preparation of this manuscript.

References and Notes

1. Reviews: Baldwin, J.; Henderson, I. *Med. Res. Rev.* **1996**, *16*, 391–405; Ellman, J.A.; Thompson, L.A. *Chem. Rev.* **1996**, *96*, 555–600; Terrett, N.K.; Gardner, M.; Gordon, D.W.; Kobylecki, R.J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135–73.
2. Phillips, G.B.; Wei, G.P. *Tetrahedron Lett.* **1996**, *37*, 4887–4890.
3. Carroll, F.I.; Coleman, M.C. *J. Med. Chem.* **1975**, *18*, 318–320.
4. Belleau, B; Martel, R; Lacasse, M; Menard, Weinberg, N.L.; Perron, Y.G. *J. Am. Chem. Soc.* **1968**, *90*, 832.
5. All nitroanilines were prepared by the method of Hunger et al. (*Helv. Chim. Acta* **1960**, *43*, 1032) and gave satisfactory spectroscopic data.
6. Yields and purities were calculated by performing pilot reactions in 4 mL preweighed vials. After reaction, vials were reweighed and the crude material dissolved in standardized 0.1M benzaldehyde in CD_3OD. Integration then provides product molarity and weight and therefore purity by ratio. The accuracy is estimated to be ±5%. Proton T_1 values were estimated for all of the components of the mixture to ensure that the interpulse delay of the ^1H NMR spectrum was long enough to allow complete proton relaxation and therefore quantitative integration. As a check on the method, the benzimidazole corresponding to etonitazene was isolated by column chromatography in 86% yield and was identical to authentic etonitazene. Also, the material from the reaction between **2A** and phenyl acetic acid was isolated in 85% yield and provided satisfactory NMR and elemental analyses.
7. Smith, P.W.; Lai, J.Y.Q.; Whittington, A.R.; Cox, B.; Houston, J.G.; Stylli, C.H.; Banks, M.N., Tiller, P.R. *Bioorg. Med. Chem. Lett* **1994**, *4*, 2821–2824.
8. Desai, M.C.; Stramiello, L.M.S. *Tetrahedron Lett.* **1993**, *34*, 7685–7688.
9. Kiso, Y; Kai, Y; Yajima, H. *Chem. Pharm. Bull.* **1973**, *21*, 2507–2510.
10. Carroll, F.I.; Handy, R.W.; Kepler, J.A.; Gratz, J.A. *J. Heterocyclic Chem* **1967**, *4*, 262–267.; Adger, B. M.; Young, R.G. *Tetrahedron Lett.* **1984**, *25*, 5219–5222.

(*Received in USA* 6 *May* 1997; *revised* 21 *May* 1997; *accepted* 29 *May* 1997)