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Tetrabutylammonium Fluoride-Mediated Rapid Alkylation Reaction in Microtiter Plates for the Discovery of Enzyme Inhibitors in Situ

Chung-Yi Wu,[a, b] Ashraf Brik,[a] Sheng-Kai Wang,[a] Yu-Hsien Chen,[a] and Chi-Huey Wong*[a, b]

We have recently developed a new strategy for the rapid identification and optimization of enzymes inhibitors in microtiter plates. This approach relies on the use of high-yield organic reactions that can be carried out in water or water-miscible nontoxic solvents on microscales without protecting groups, so that the product can be assayed directly in situ without isolation or purification. Using this approach, one can quickly modify a lead compound with a small set of building blocks to identify a potent inhibitor. For example, using amide- and triazole-forming reactions, we have discovered potent inhibitors against HIV protease,[1] SARS 3CL protease,[2] α-fucosidase,[3] sulfotransferase,[4] and α-1,3-fucosyltransferase.[5] In order to expand the scope of this approach, we report here the development of tetrabutylammonium fluoride (TBAF)-mediated alkylation in microtiter plates for the identification of potent inhibitors in situ against several enzymes including cathepsin B, arylsulfotransferase, and HIV protease.

[a] *Dr. C.-Y. Wu, Dr. A. Brik, S.-K. Wang, Y.-H. Chen, Prof. C.-H. Wong*
Department of Chemistry and the Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2409
E-mail: wong@scripps.edu

[b] *Dr. C.-Y. Wu, Prof. C.-H. Wong*
The Genomics Research Center, Academia Sinica
No. 128 Academia Road Section 2, Nan-Kang, Taipei, 11529 (Taiwan)

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TBAF-mediated alkylation of carboxylates and nucleoside bases under anhydrous conditions has been reported, in which TBAF was used as catalyst[6] or as stoichiometric reagent.[7] We have recently developed a method of TBAF-mediated selective alkylation of purines at N-9 in aqueous solution and applied the methodology to combinatorial synthesis in microtiter plates for use in in situ screening to identify potent sulfotransferase inhibitors.[8] In this study, we have further extended this strategy to TBAF-mediated alkylation of carboxylates, sulfonamide nitrogen, secondary amines, and the *N*-hydroxyl group of triazoles, and demonstrated its utility in drug discovery. In our initial study on ester formation, we found that on using commercially available TBAF (1 M in THF) as a stoichiometric reagent without any additional solvent added, the reaction between acids and alkyl halides gave the corresponding ester in high yield. As an example, treatment of benzoic acid with 1.2 equiv of TBAF (1 M in THF) and 1.2 equiv of benzyl bromide at room temperature for 30 min gave rise to the corresponding benzyl ester quantitatively. We then prepared 1 M TBAF in different solvents (Table 1) and found that the reaction can be carried out efficiently in these solvents system as well. Interestingly, the product was also isolated in excellent yields when water was used as solvent, though a longer reaction time was required for completion.

Table 1. Reaction of benzoic acid with benzyl bromide in the presence of TBAF and different solvents.[a]

PhCOOH + BnBr $\xrightarrow{\text{TBAF}}$ PhCOOBn + HBr

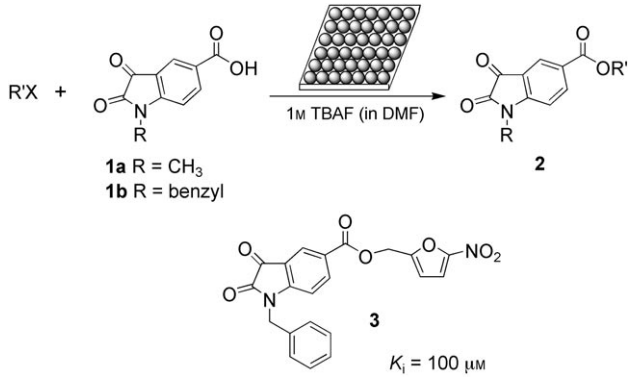
Solvent	Time	Yield of PhCOOBn [%][b]
THF	30 min	96
DMF	30 min	97
DMSO	30 min	96
CH_2Cl_2	30 min	97
dioxane	30 min	93
CH_3CN	30 min	96
C_6H_6	6 h[c]	91
H_2O	6 h[c]	92

[a] Reaction conditions: Benzoic acid (1 mmol), benzyl bromide (1.1 mmol), TBAF (1.1 mmol), room temperature. [b] Isolated yield. [c] Stir vigorously.

To examine the scope and efficiency of the TBAF-assisted esterification reaction, several acids and alkyl halides were used (Table 2). Our results showed that most of these reactions went to completion within 4 hours and that the product was isolated in a very good yield. Secondary alkyl halides, such as *iso*-propyl iodide require higher temperature and more time for completion (entry 5). Interestingly, no restrictions were found with regard to the nature of carboxylic acids; triphenylacetic acid and *tert*-butylacetic acid, for example, do not affect the reaction efficiency and could be transformed successfully to the corresponding esters (entries 12–15). Moreover, esterification of β-unsaturated carboxylic acids and α-substituted acids proceeded well, and no isomerization was observed (entries 16, 17, 20). Selective transformation of the carboxylic acid in the presence of other functional groups to the corresponding ester was also successful (entries 21–26). Notably, polyhydroxy acids, such as shikimic acid and quinic acid proceeded well, and the corresponding benzyl esters were isolated in good yields (entries 27 and 28).

The broad scope, efficiency, and reliability of TBAF-assisted esterification permitted us to apply it in microtiter plates followed by in situ screening. Isatin analogues based on core **1** are efficient inhibitors against caspases 3[9] and rhinovirus 3C protease.[10] Cathepsin B is a cysteine protease that operates by a similar mechanism. This enzyme has been a target for drug discovery due to its association with a number of diseases, such as metastatic tumors.[11] However, there are only a few non-peptidic small molecules with K_i values in the μM range that have been reported so far, thus development of new inhibitors is of current interest.

We selected the isatin core **1** ($K_i > 5$ mM) as the starting point to create a library in microtiter plates around the acid functionality. More than 150 compounds were generated from 78 different alkyl halides[12] and cores **1a** and **1b** (Scheme 1). The reactions were complete within 4 h, as analyzed by TLC and LC-MS, and gave the desired ester compounds as the only products. Each well was diluted to 3 mM and directly screened against cathepsin B (from bovine spleen) as previously described.[11] Wells that inhibited 50% of cathepsin B activity were diluted to 200 μM for further assay, and compound **3** emerged as the best inhibitor from this library. Compound **3** was isolated, purified, and characterized, and was determined to be a competitive inhibitor with $K_i = 100$ μM. We also sought to improve enzyme inhibition through replacement of the isatin group in compound **3**. To do this, a "reverse library" was synthesized that contained **5**, in which the 2-(bromomethyl)-5-



Scheme 1. The reaction of 5-carboxyl-isatin (**1**) with a library of 78 organic halides for the subsequent high-throughput in situ screening of cathepsin B inhibition.

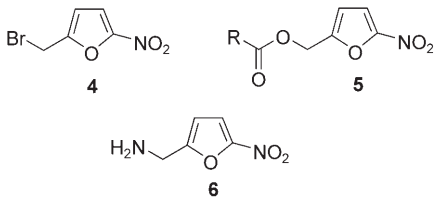
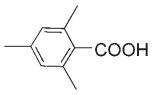
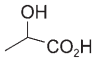
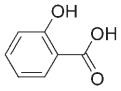
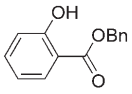
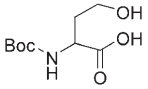
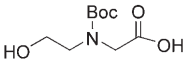
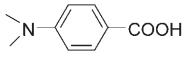
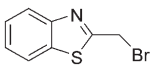
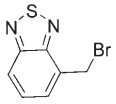
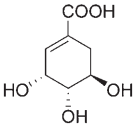
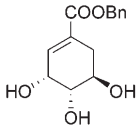
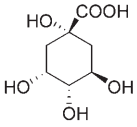
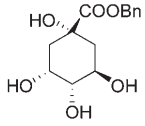


Table 2. TBAF-assisted esterification of carboxylic acids with alkyl halides.

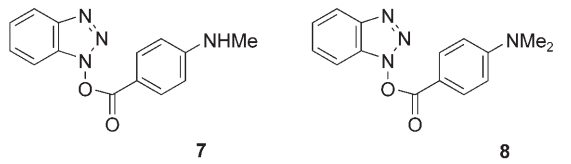
	RCO_2H	R′X	Reaction time [h]	Yield of ester[b] [%]	
1	$PhCO_2H$	MeI	0.5	96	
2		EtI	0.5	93	
3		$(CH_3)_2CH(CH_2)_2CH_2Br$	1	95	
4		$(CH_3)_2CHCH_2Br$	1	95	
5[c]		*i*PrI	4	82	
6[c]		$(CH_3)_3Cl$	24	0	
7[c]		$(CH_3)_3CCH_2I$	24	0	
8		$CH_2{=}CHCH_2Br$	0.5	91	
9		$HC{\equiv}CCH_2Br$	0.5	90	
10	$CH_3(CH_2)_7CO_2H$	$PhCH_2Br$	0.5	95	
11	$CH_3(CH_2)_7CO_2H$	MeI	1	93	
12	*t*BuCO_2H	$PhCH_2Br$	1.5	95	
13	*t*BuCO_2H	MeI	2	92	
14	Ph_3CCO_2H	$PhCH_2Br$	3	94	
15	Ph_3CCO_2H	MeI	2	92	
16	*trans*-$PhCH{=}CHCO_2H$	$PhCH_2Br$	0.5	95	
17	*trans*-$PhCH{=}CHCO_2H$	MeI	1	94	
18		$PhCH_2Br$	1.5	95	
19		MeI	1	95	
20		$PhCH_2Br$	2.5	59	
21		$PhCH_2Br$	12	83	
22		MeI	15	70	
23		MeI	1.5	86	
24		$PhCH_2Br$	4	83	
25			1	85	
26			1	89	
27[d]		$PhCH_2Br$	2.5	87	
28[d]		$PhCH_2Br$	3.5	91	
29	$Br(CH_2)_4CO_2H$	×	1	95	

[a] Unless other specified, the reaction was carried out with TBAF (1.2 equiv, 1 M in THF) and alkyl halides (1.2 equiv) at room temperature. [b] Isolated yield. [c] At 55 °C. [d] TBAF (1.2 equiv, 1 M in DMF).

nitrofuran core group **4** was treated with 84 different acids[12] by TBAF-assisted ester formation, then diluted to 20 μM and directly screened against cathepsin B. Unfortunately, no inhibitor was found. Then, we used 2-(aminomethyl)-5-nitrofuran (**6**), 84 acids and (1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) to form the amide compounds. None of the amide compounds showed inhibitory activities toward cathepsin B at 20 μM concentration. However, from the

HBTU-mediated formation of benzotriazole esters, compounds **7** and **8** were identified as the best inhibitors (Scheme 2), with IC_{50} values of 8 and 10 μM, respectively. These compounds are very stable, and no decomposition was observed at pH 5.0–8.0 over 24 h at room temperature. Compounds **7** and **8** are irreversible inactivators of the enzyme (detailed enzyme behavior is shown in the Supporting Information), with inactivation constants $k_{inact} = 0.7 \times 10^{-3}$ and $1.1 \times 10^{-3}\ s^{-1}$ and inhibition constants $K_i = 7.35$ and 7.18 μM, respectively.

To further extend the scope of TBAF-assisted reactions, we selected sulfonamide **9**, benzotriazole (**10**), and 1-hydroxybenzotriazole (HOBt; **11**) as core compounds. We found these compounds were also very sensitive to *N*- (**9**, **10**) or *O*-alkylation (**11**) in the TBAF-assisted reaction. Compound **9** showed



Scheme 2. Benzotriazole esters **7** and **8**, identified through HBTU-mediated ester formation, are irreversible inhibitors of cathepsin B with K_i values of 7.35 and 7.18 μM, respectively.

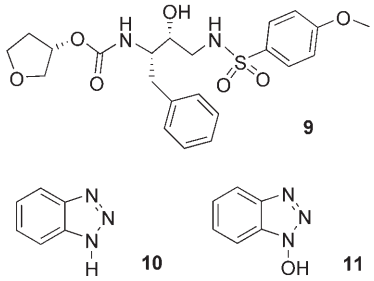
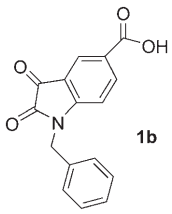
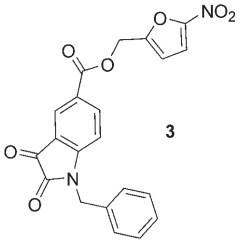
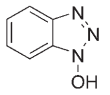
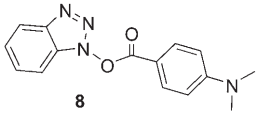
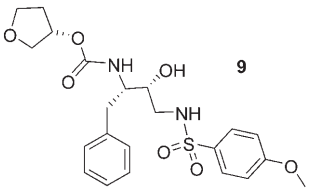
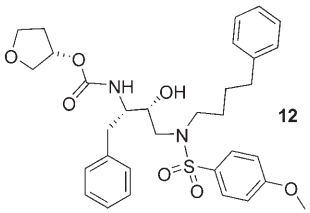
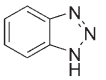
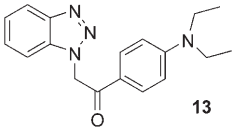
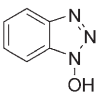
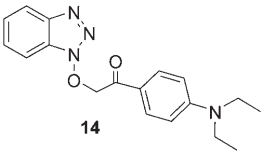
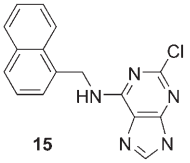
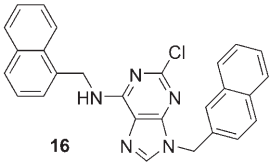


Table 3. Summary of reaction types and inhibitors found against different enzymes.

Core	Library used	Reaction type	Inhibitor	Target enzyme	K_i
 **1b**	organic halides	esterification	 **3**	cathepsin B	100 μM
 **11**	carboxylates	esterification	 **8**	cathepsin B	7.18 μM[a]
 **9**	organic halides	*N*-alkylation	 **12**	HIV protease	1.1 nM
 **10**	organic halides	*N*-alkylation	 **13**	cathepsin B	125 μM
 **11**	organic halides	*O*-alkylation	 **14**	cathepsin B	44 μM
 **15**	organic halides	*N*-alkylation	 **16**	aryl-sulfotransferase	9 nM[b]

[a] Irreversible inactivator. [b] See ref. [8].

alkylation at the sulfonamide nitrogen exclusively, no *N*-alkylation was found at the amide nitrogen. Benzotriazole **10** was alkylated at N-1 exclusively. With these results in hand, compounds **9**–**11** were treated with the same 78 organic halides and TBAF in microtiter plates with small stirring bar (2 mm in length) at room temperature for 6 h; then, after dilution, assayed directly against HIV protease or cathepsin B. The inhibitors with the highest activity were synthesized, purified, and characterized. Compound **12** showed over 1000 times more activity for HIV protease than core compound **9** ($K_i > 1$ μM) with $K_i = 1.1$ nM. Compounds **13** and **14** were new-type nonpeptidic reversible competitive inhibitors against cathepsin B with $K_i =$ 125 and 44 μM, respectively. Table 3 summarizes the results of TBAF-mediated alkylation and enzyme assays.

In conclusion, we have discovered that TBAF-mediated alkylation is an efficient, mild, and reliable approach for the rapid synthesis of esters and alkyl derivatives of secondary amines and sulfonamides. This chemistry is amenable to microtiter plates for library preparation followed by in situ screening without isolation or purification, and using this approach new inhibitors of cathepsin B and HIV protease have been discovered. Work is in progress to identify other organic reactions applicable to this approach.

Acknowledgements

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Keywords: alkylation · combinatorial chemistry · esterification · inhibitors · TBAF

[1] a) A. Brik, Y.-C. Lin, J. Elder, C.-H. Wong, *Chem. Biol.* **2002**, *9*, 891 – 896; b) T.-J. Cheng, A. Brik, C.-H. Wong, C.-C. Kan, *Antimicrob. Agents Chemother.* **2004**, *48*, 2437 – 2447; c) A. Brik, J. Muldoon, Y.-C. Lin, J. Elder, D. S. Goodsell, A. J. Olson, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *ChemBioChem* **2003**, *4*, 1246 – 1248.

[2] C.-Y. Wu, J.-T. Jan, S.-H. Ma, C.-J. Kuo, H.-F. Juan, Y.-S. E. Cheng, H.-H. Hsu, H.-C. Huang, D. Wu, A. Brik, F.-S. Liang, R.-S. Liu, J.-M. Fang, S.-T. Chen, P.-H. Liang, C.-H. Wong, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10012 – 10017.

[3] a) C.-Y. Wu, C.-F. Chang, J. S.-Y. Chen, C. H. Wong, C.-H. Lin, *Angew. Chem.* **2003**, *115*, 4809 – 4912; *Angew. Chem. Int. Ed.* **2003**, *42*, 4661 – 4664; b) C.-F. Chang, C.-W. Ho, C.-Y. Wu, T.-A. Chao, C.-H. Wong, C.-H. Lin, *Chem. Biol.* **2004**, *11*, 1301 – 1306.

[4] M. D. Best, A. Brik, E. Chapman, L. V. Lee, W.-C. Cheng, C.-H. Wong, *ChemBioChem* **2004**, *5*, 811 – 819.

[5] L. V. Lee, M. L. Mitchell, S.-J. Huang, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 9588 – 9589.

[6] a) G. Bram, C. Decodts, *Synthesis* **1985**, 543 – 545; b) T. Ooi, H. Sugimoto, K. Doda, K. Maruoka, *Tetrahedron Lett.* **2001**, *42*, 9245 – 9248.

[7] K. K. Ogilvie, S. L. Beaucage, M. F. Gillen, *Tetrahedron Lett.* **1978**, *19*, 1663 – 1666.

[8] A. Brik, C.-Y. Wu, M. D. Best, C.-H. Wong, *Bioorg. Med. Chem.* **2005**, *13*, 4622 – 4626.

[9] D. Lee, S. A. Long, J. H. Murray, J. L. Adams, M. E. Nuttall, D. P. Nadeau, K. Kikly, J. D. Winkler, C. M. Sung, M. D. Ryan, M. A. Levy, P. M. Keller, W. E. Dewolf, *J. Med. Chem.* **2001**, *44*, 2015 – 2026.

[10] S. E. Webbwe, J. Tikhe, S. T. Worland, S. A. Fuhrman, T. F. Hendrickson, D. A. Matthews, R. A. Love, A. K. Patick, J. W. Meador, R. A. Ferre, E. L. Brown, D. M. Delisle, C. E. Ford, S. L. Binford, *J. Med. Chem.* **1996**, *39*, 5072 – 5082.

[11] I. K. Lim, S. O. Meroueh, M. Lee, M. J. Heeg, S. J. Mobashery, *J. Am. Chem. Soc.* **2004**, *126*, 10271 – 10277, and references cited therein.

[12] The structures of 78 organic halides and 84 acids are given in the Supporting Information.

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