ORGANIC LETTERS

2007
Vol. 9, No. 26
5617−5620

Amino Acid Derived Enamides: Synthesis and Aminopeptidase Activity

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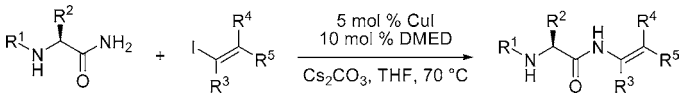
Received October 22, 2007

ABSTRACT



Recently developed copper-catalyzed coupling methodology has been applied to the synthesis of amino acid derived enamides. Bond formation proved to be strongly influenced by protection strategy and vinyl iodide substitution while tolerant of limited side chain functionality. Assessment of aminopeptidase activity revealed a preference for (*E*)-1,2-disubstituted constructs.

Interest in the enamide functionality has increased exponentially in recent years due primarily to the expanding number of biologically relevant molecules in which it is found.[1] The chemo- and stereoselectivity challenges associated with enamide synthesis in these complex molecular frameworks has underscored the limitations of conventional methods, particularly the acylation of imines and ketoximes,[2] direct condensation of aldehydes and amides,[3] Curtius rearrangement of α,β-unsaturated acyl azides,[4] dehydration of hemiaminals,[5] and a variety of olefination processes.[6] In response, the synthetic community has rapidly developed a variety of mild, metal-mediated C−N bond-forming processes,[7] including Ag-[8] and Ru-catalyzed hydroamidation,[9] Pd-catalyzed oxidative amidation of olefins,[10] and direct Pd-[11] and Cu-catalyzed[12] coupling of amides with vinyl (pseudo) halides.[13]

Our own interest evolved from a desire to integrate an alternative pro-drug system within a substrate peptide designed to target matrix metalloproteinases (MMPs).[14] In

(1) For selected total syntheses of the salicylate enamide macrolides, see: (a) Furstner, A.; Dierkes, T.; Thiel, O. R.; Blanda, G. *Chem. Eur. J.* **2001**, *7*, 5286. (b) Nicolaou, K. C.; Kim, D. W.; Baati, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 3701. (c) Wang, X.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2003**, *125*, 6040. For a recent review, see: Yet, L. *Chem. Rev.* **2003**, *103*, 4283.

(2) Recent examples: (a) Sun, C.; Weinreb, S. M. *Synthesis* **2006**, 3585. (b) Savarin, C. G.; Boice, G. N.; Murry, J. A.; Corley, E.; DiMichele, L.; Hughes, D. *Org. Lett.* **2006**, *8*, 3903. For a recent application in the synthesis of salicylihalamide A, see: Smith, A. B., III; Zheng, J. *Tetrahedron* **2002**, *58*, 6455.

(3) (a) Zeeza, C. A.; Smith, M. B. *Synth. Commun.* **1987**, *17*, 729. (b) Kiefel, M. J.; Maddock, J.; Pattenden, G. *Tetrahedron Lett.* **1992**, *33*, 3227.

(4) (a) Brettle, R.; Mosedale, A. J. *J. Chem. Soc., Perkin. Trans. 1* **1988**, *2*, 185. (b) Snider, B. B.; Song, F. *Org. Lett.* **2000**, *2*, 407. (c) Stefanuti, I.; Smith, S. A.; Taylor, R. J. K. *Tetrahedron Lett.* **2000**, *41*, 3735. (d) Kuramochi, K.; Watanabe, H.; Kitahara, T. *Synlett* **2000**, 397. For a recent application in the total synthesis of salicylihalamide A, see: Wu, Y.; Liao, X.; Wang, R.; Xie, X.-S.; De Brabander, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 3245.

(5) (a) Wang, X.; Porco, J. A., Jr. *J. Org. Chem.* **2001**, *66*, 8215. (b) Bayer, A.; Maier, M. E. *Tetrahedron* **2004**, *60*, 6665.

(6) (a) Peterson: (i) Hudrlick, P. F.; Hudrlick, A. M.; Rona, R. J.; Misra, R. N.; Withers, G. P. *J. Am. Chem. Soc.* **1977**, *99*, 1993. (ii) Cuevas, J.-C.; Patil, P.; Snieckus, V. *Tetrahedron Lett.* **1989**, *30*, 5841. (iii) Palomo, C.; Aizpurua, J. M.; Legido, M.; Picard, J. P.; Dunogues, J.; Constantieux, T. *Tetrahedron Lett.* **1992**, *33*, 3903. (iv) Furstner, A.; Brehm, C.; Cancho-Grande, Y. *Org. Lett.* **2001**, *3*, 3955. (b) Horner−Wittig: Couture, A.; Deniau, E.; Grandclaudon, P. *Tetrahedron Lett.* **1993**, *34*, 1479. (c) Wadsworth−Emmons: (i) Paterson, I.; Cowden, C.; Watson, C. *Synlett* **1996**, 209. (ii) Villa, M. V. J.; Targett, S. M.; Barnes, J. C.; Whittingham, W. G.; Marquez, R. *Org. Lett.* **2007**, *9*, 1631.

(7) For a recent review, see; Dehli, J. R.; Legros, J.; Bolm, C. *Chem. Commun. (Cambridge, UK)* **2005**, 973.

(8) Sun, J.; Kozmin, S. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4991.

(9) (a) Kondo, T.; Tanaka, A.; Kotachi, S.; Watanabe, Y. *J. Chem. Soc., Chem. Commun.* **1995**, 413. (b) Goossen, L. J.; Rauhaus, J. E.; Deng, G. *Angew. Chem., Int. Ed.* **2005**, *44*, 4042.

(10) (a) Hosokawa, T.; Takano, M.; Kuroki, Y.; Murahashi, S.-Y. *Tetrahedron Lett.* **1992**, *33*, 6643. (b) Brice, J. L.; Meerdink, J. E.; Stahl, S. S. *Org. Lett.* **2004**, *6*, 1845. (c) Hansen, A. L.; Skrydstrup, T. *J. Org. Chem.* **2005**, *70*, 5997. (a) Lee, J. M.; Ahn, D.-S.; Jung, D. Y.; Lee, J.; Do, Y.; Kim, S. K.; Chang, S. *J. Am. Chem. Soc.* **2006**, *128*, 12954.

(11) (a) Kozawa, Y.; Mori, M. *Tetrahedron Lett.* **2002**, *43*, 111. (b) Wallace, D. J.; Klauber, D. J.; Chen, C.-y.; Volante, R. P. *Org. Lett.* **2003**, *5*, 4749. (c) Willis, M. C.; Brace, G. N.; Holmes, I. P. *Synthesis* **2005**, 3229. (d) Klapars, A.; Campos, K. R.; Chen, C.-y.; Volante, R. P. *Org. Lett.* **2005**, *7*, 1185. (e) Barluenga, J.; Moriel, P.; Aznar, F.; Valdes, C. *Org. Lett.* **2007**, *9*, 275.

10.1021/ol7025729 CCC: $37.00
Published on Web 11/28/2007

principle, the enamide function was expected to append the localization sequence in such a manner that the combination of endo- and aminopeptidase (APM) action would reveal a carbonyl fragment on the drug substance (Figure 1). In this

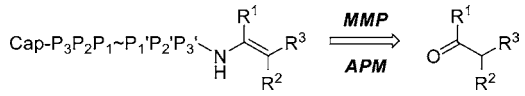
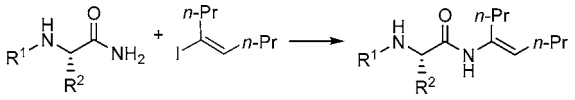


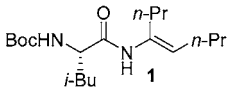
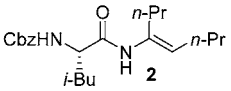
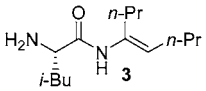
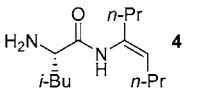
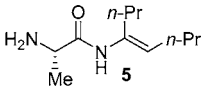
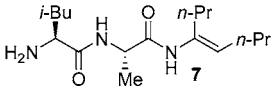
Figure 1. Enamide-based pro-drug strategy.

regard, a principal requirement of the design was compatibility with essential enzyme systems such that maximum turnover in the target tissue was achieved. We therefore required a fast and flexible synthesis of amino acid derived enamides[15] in order to evaluate factors influencing the enzyme kinetics. As such, we decided to investigate the utility of modern Cu-catalyzed amidation methodology[16] and report herein our results on the scope and limitations in the C−N bond-forming process and include an evaluation of proteolysis activity of these constructs with aminopeptidase M.

Our study commenced with an evaluation of the impact of protection strategy on the efficiency and chemoselectivity of C−N bond formation. Using the standard conditions reported by Buchwald[17] and the model vinyl iodide, (*E*)-4-iodooct-4-ene, we were pleased to observe the efficient and completely regioselective formation of the expected enamide derivative of Boc-Leu-NH_2 (entry 1, Table 1). Reaction of

Table 1. Effect of Protection Strategy on Coupling Efficiency



entry	product[a]	yield[b](convn)[c]	er[g]
1	 **1**	90(98) 78(89)[d]	99.7/0.3
2	 **2**	54(82)[e]	99.8 / 0.2 66.6/33.4
3	 **3**	35(37)	
4	 **4**	5(15)	
5	 **5**	34(34)	99.5/0.5
6	 **7**	54(61)[f]	Ala - 99.5/0.5 Leu - 99.7/0.3

[a] Conditions: 5 mol % of CuI, 10 mol % of *N*,*N*′-dimethylethylenediamine, 2 equiv of amide, 1.5 equiv of Cs_2CO_3, 0.5 M THF, 70 °C, 16 h. Reaction times not optimized. Average of at least two runs. [b] Isolated yield after silica gel chromatography. [c] Conversion determined by HPLC analysis of the crude reaction mixture. [d] 1 equiv of amide. [e] Isolated 26% of the derived hydantoin. [f] 0.25 M. [g] Determined by chiral GLC analysis (see the Supporting Information for details).

the analogous Cbz-protected material, however, resulted in formation of a 2:1 mixture of the expected product and its derived hydantoin (entry 2).[18] *Importantly, we observed that protection of the* α*-nitrogen was not required, as the free amino enamide could be isolated as the exclusive product*, in accordance with conversion (entries 3 and 5). Equally impressive was reaction with the dipeptide fragment H-Leu-Ala-NH_2, which revealed complete chemoselectiviy of C−N bond formation (entry 6). In contrast, very low conversion was noted with the corresponding (*Z*)-4-iodooct-4-ene (entry 4).[19]

Our next concern was to address the influence of vinyl iodide substitution and stereochemistry on coupling ef-

(12) (a) Ogawa, T.; Kiji, T.; Hayami, K.; Suzuki, H. *Chem. Lett.* **1991**, 1443. (b) Shen, R.; Porco, J. A., Jr. *Org. Lett.* **2000**, *2*, 1333. (c) Jiang, L.; Job, G. E.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 3667. (d) Lam, P. Y. S.; Vincent, G.; Bonne, D.; Clark, C. G. *Tetrahedron Lett.* **2003**, *44*, 4927. (e) Coleman, R. S.; Liu, P.-H. *Org. Lett.* **2004**, *6*, 577. (f) Han, C.; Shen, R.; Su, S.; Porco, J. A., Jr. *Org. Lett.* **2004**, *6*, 27. (g) Pan, X.; Cai, Q.; Ma, D. *Org. Lett.* **2004**, *6*, 1809. (h) Hu, T.; Li, C. *Org. Lett.* **2005**, *7*, 2035. (i) Lu, H.; Li, C. *Org. Lett.* **2006**, *8*, 5365. (j) Martin, R.; Rivero, M. R.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 7079. (k) Yuan, X.; Xu, X.; Zhou, X.; Yuan, J.; Mai, L.; Li, Y. *J. Org. Chem.* **2007**, *72*, 1510.

(13) For closely related methods which involve the preparation of ynamides and their subsequent conversion to enamides, see: (a) Witulski, B.; Buschmann, N.; Bergstrasser, U. *Tetrahedron* **2000**, *56*, 8473. (b) Tanaka, R.; Hirano, S.; Urabe, H.; Sato, F. *Org. Lett.* **2003**, *5*, 67. (c) Dunetz, J. R.; Danheiser, R. L. *Org. Lett.* **2003**, *5*, 4011. (d) Zhang, X.; Zhang, Y.; Huang, J.; Hsung, R. P.; Kurtz, K. C. M.; Oppenheimer, J.; Petersen, M. E.; Sagamanova, I. K.; Shen, L.; Tracey, M. R. *J. Org. Chem.* **2006**, *71*, 4170 and references therein. For catalytic olefin isomerization methodology, see: (e) Trost, B. M.; S. J.-P. *Angew. Chem., Int. Ed.* **2001**, *40*, 1468. (f) Sergeyev, S. A.; Hesse, M. *Helv. Chim. Acta* **2003**, *86*, 750. For a unique rearrangement of α-silyl amides, see: (g) Lin, S.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 512. (h) Zhang, X.; Houck, K. N.; Lin, S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 5111.

(14) Harris, T. D.; Yalamanchili, P.; Cesati, R. R., III. Compounds Containing Matrix Metalloproteinase Substrates and Methods of their use. EP1691845 A1, August 23, 2006.

(15) Several papers addressing enamide formation in the context of amino amides have recently appeared: (a) Yuen, J.; Fang, Y.-Q.; Lautens, M. *Org. Lett.* **2006**, *8*, 653. (b) He, G.; Wang, J.; Ma, D. *Org. Lett.* **2007**, *9*, 1367. (c) Toumi, M.; Couty, F.; Evano, G. *Angew. Chem., Int. Ed.* **2007**, *46*, 572. (d) Toumi, M.; Couty, F.; Evano, G. *J. Org. Chem.* **2007**, *72*, 9003.

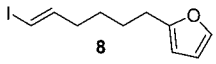
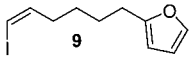
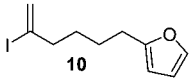
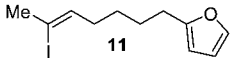
(16) Given the requirement of activating functionality in current Pd-catalyzed processes, we opted for the Cu-catalyzed amidation methods such that our drug substance would be released with minimal trace of the enamide linkage. It should be noted, however, that Willis and co-workers have recently reported the Pd-catalyzed amidation of simple cyclic pseudohalides (ref 11c); extension to acyclic substrates has yet to be reported.

(17) (a) Reference 12c. (b) For purposes of comparison, we also evaluated the methods of Porco[12b] (98% conversion and 70% yield with 20 mol % of CuTC) and Ma[12g] (88% conversion and 73% yield with 10 mol % of CuI and 20 mol % *N*,*N*′-dimethylglycine; 37% conversion and 28% yield with 5 mol % of CuI and 10 mol % of *N*,*N*′-dimethylglycine) in the reaction of H-Leu-NH_2 with (*E*)-4-iodooct-4-ene (e.g., entry 3, Table 1).

(18) Control experiments indicate hydantoin formation occurs *after* the cross-coupling event.

ficiency. Using four model vinyl iodides, we noted a dramatic enhancement in reaction rate with the (*E*)-1,2-disubstituted derivative (entry 1, Table 2). As comparison of the data in

Table 2. Affect of Vinyl Iodide Substitution and Stereochemistry

entry	vinyl iodide	yield(convn)[a]	er[c]
1	 **8**	87(98)	97.9/2.1
2	 **9**	50(62)	99.1/0.9
3	 **10**	20(47)[b]	
4	 **11**	41(51)	99.7/0.3

[a] Conditions: 5 mol % of CuI, 10 mol % of *N*,*N*′-dimethylethylenediamine, 2 equiv of H-Leu-NH_2, 1.5 equiv of Cs_2CO_3, THF, 70 °C, 16 h. Average of at least two runs. [b] Mass balance consists of a 1:1 mixture of imidazolidinone diastereomers resulting from cyclization of the parent enamide. [c] Determined by chiral GLC analysis (see the Supporting Information for details).

Table 2 reveals, alternative substitution patterns on the vinyl iodide typically resulted in reduced conversion; coupling efficiency did not, however, markedly decrease. During the reaction with 2,2-disubstitutued vinyl iodide **10**, we observed formation of the derived imidazolidinone in addition to the expected enamide product (entry 3, Table 2), a side reaction unique to this vinyl iodide substitution pattern.

In the design of our pro-drug linker, the *C*-terminal amino acid serves as part of the peptide substrate as well as the release mechanism and must therefore be tolerated by both the endo- and aminopeptidases. In this context, we decided to evaluate several amino acid derivatives of our (*E*)-1,2-disubstituted vinyl iodide **8** in order to identify a series of residues which maintain high aminopeptidase activity and provide diversity in selection of the MMP substrate. Hence, based on known substrate specificity of the target MMPs, a series of amino amides were selected and coupled, *without protecting groups*, to **8** using the standard Buchwald conditions described above.

As the results in Table 3 indicate, a variety of side chain functionality was well tolerated by the catalyst system. Overall reaction rates and yields were highest with lipophilic side chains, including methionine (entries 2−6), although *β*-substitution resulted in a moderate reduction of isolated yield (e.g., entries 3 and 5). In certain cases limited solubility of the amino amide required the reaction to be run in DMF

(19) (*Z*)-4-Iodooct-4-ene was prepared from 4-octyne according to a popular published method: Kamiya, N.; Chikami, Y.; Ishii, Y. *Synlett* **1990**, 675. We noted, however, the stereochemistry of HI addition was incorrectly assigned in this report; see the Supporting Information for complete details.

(20) For comparison, the product of **8** and H-Phe-NH_2 was isolated in only 54% yield using these conditions.

Table 3. Amino Acid Substitution and Hydrolysis Rate

entry	side chain[a]	yield (convn) (%)	rate (μmol/min·U)[e]
1	Gly (**12**)	32 (53)	<0.050
2	Ala (**13**)	82 (98)	1.83 (0.950)
3	Val (**14**)	58 (80)	<0.050
4	Leu (**15**)	87 (98)	0.411 (0.401)
5	Ile (**16**)	63 (91)	<0.050
6	Met (**17**)	77 (98)	0.846 (0.711)
7	Gln[b] (**18**)	31 (95)[c]	0.245 (0.252)
8	Phe (**19**)	87 (98)	0.416 (0.350)
9	Tyr[b] (**20**)	30 (78)	0.396 (0.353)
10	Trp (**21**)	50 (98)[d]	<0.05

[a] Conditions: 5 mol % of CuI, 10 mol % of *N*,*N*′-dimethylethylenediamine, 1 equiv of **8**, 2 equiv of amino amide, 1.5 equiv of Cs_2CO_3, THF, 70 °C, 16 h. Average of at least two runs. [b] 0.5 M DMF, 50 °C, 1 h. [c] Also isolated 7% *γ*-lactam- and 7% derived dimeric-coupled products. [d] Also isolated 10% indole-coupled and 20% dimeric products. [e] See Table 4 for assay details.

solution. The dramatic rate acceleration observed with this solvent system resulted in an overall reduction in coupling efficiency[20] (e.g., entries 7 and 9). Noteworthy among these examples was the chemoselectivity in the coupling of H-Gln-NH_2, where a 20:1 preference for the main chain amide was observed; perhaps reflecting a directing influence of the free α-nitrogen. With regard to other competing side chain functionality, we observed significant reaction at the indole nitrogen (entry 10) as well as a distinct preference for coupling at the phenol of H-Tyr-NH_2 (entry 9). Other amino amides which proved problematic in this regard include H-Arg-NH_2, H-His-NH_2, and H-Glu-NH_2 (data not shown).

The measured enantiomeric ratio for several enamide contructs revealed little to no loss of stereochemical intergrity during the coupling reaction (cf. Tables 1 and 2). That said, the derived hydantoin product of **2** was shown to be nearly racemic. We did, however, note an erosion of enantiomeric ratio at elevated concentration, where coupling of H-Leu-Ala-NH_2 required more dilute conditions (0.25 M), as reaction at 0.50 M produced a 4:1 mixture of diastereomeric products at the alanine α-carbon. It is interesting to note that no stereomutation was ever observed at the leucine residue of this dipeptide.

With a series of amino acid derived enamides in hand, we turned our attention to the determination of proteolysis rates with aminopeptidase M (Tables 3 and 4). In the experiment, substrates were incubated with two unique enzyme concentrations (25 min at 37 °C) and the change in peak area monitored by HPLC. Rates were calculated according to following equation

$$k = \{(\%\ \text{hydrolyzed}/100)*[S]\}/[E]*[\text{time}]$$

where

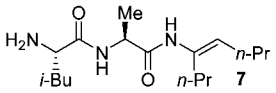
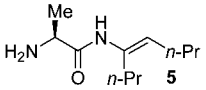
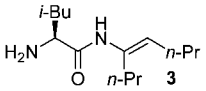
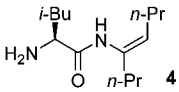
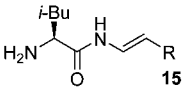
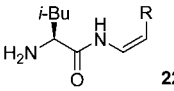
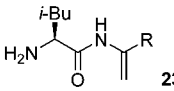
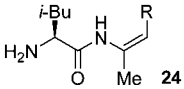
S = test substrate concentration in μmol

E = aminopeptidase concentration in U/mL

k = μmol substrate hydrolyzed/minute/U

For purposes of comparison, we first determined the hydrolysis rate of the *N*-terminal residue on dipeptide enamide **7** (entry 1, Table 4).[21] Subsequent evaluation of the

Table 4. Hydrolysis of *N*-Terminal Residue by APM

entry	enamide	rate (μmol/min•U)[a]
1	 **7**	1.63 (1.67)
2	 **5**	0.048 (0.109)
3	 **3**	<0.050
4	 **4**	<0.050
5	 **15**	0.449 (0.432)
6	 **22**	0.112 (0.083)
7	 **23**	0.097 (0.076)
8	 **24**	<0.050

[a] The APN assay was performed at three enzyme concentrations: 0, 6.5×10^{-4} and 15.0×10^{-3} U. The rate data are given at the 6.5×10^{-4} U concentration. The value obtained at 15.0×10^{-3} U is listed in parentheses. Enzyme was denatured with MeCN due to the acid-sensitivity of the substrate. Average of two runs. R = $(CH_2)_4$(2-furan).

mono amino acid enamides derived from trisubstituted vinyl iodides revealed significantly reduced hydrolysis rates; in fact, only the alanine derivative **5** displayed measurable hydrolysis (cf. entries 2−4 and 8). Extension to the disubstituted analogues **15** and **22**−**24** revealed (*E*)-1,2-susbtitution to be well tolerated, while a dramatic reduction in rate was observed for the (*Z*)-1,2- and 2,2-disubstituted derivatives; reflecting changes in rotamer population as a consequence of developing allylic strain in these systems (e.g., entries 5−7). A trend that may limit the scope of carbonyl functionality on our drug substance.

Subsequent study of the relationship between side-chain functionality and aminopeptidase activity revealed that aliphatic side chains which do not contain a β-substituent are in general well-tolerated by the enzyme system (e.g., entries 1−6, Table 3). Also worthy of note was the dramatic enhancement in hydrolysis rate upon substitution of Ala for Gly, where hydrolysis of the alanine enamide occurs with nearly the same facility as the peptide bond in **7** (entries 1−2); the facility of this process augurs well for our pro-drug application. Interestingly, in the series of aromatic amino enamides, both Phe and Tyr were well tolerated while very little hydrolysis was noted with the Trp derivative. Taken together, these results expose several options for application as the *C*-terminal residue in our MMP substrate peptides.

In conclusion, we have described an extension of the Cu-catalyzed coupling of amides and vinyl iodides to include amino acid derived products. The expanded understanding of protecting and side chain functional group tolerance of the method, revealed herein, should find widespread application in a variety of synthetic endeavors. Further extension of the utility of an enamide-derived pro-drug strategy is a subject of continued investigation.

Acknowledgment. We are grateful to Wendy Mello, John Pietryka, Valerie Letsou, and Peta Ryan of the BMS-MI Pharmacy and Anaytical Development group for NMR and MS assistance, respectively.

Supporting Information Available: Full experimental procedures and characterization data for all new compounds described in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

OL7025729

(21) For comparison to literature values, see: Niven, G. W.; Holder, S. A.; Strøman, P. *Appl. Microbiol. Biotechnol.* **1995**, *44*, 100.