Organic Process Research & Development

Article

pubs.acs.org/OPRD

Lipase Catalyzed Regioselective Lactamization as a Key Step in the Synthesis of *N*-Boc (2*R*)-1,4-Oxazepane-2-Carboxylic Acid

Carl-Johan Aurell,* Staffan Karlsson, Fritiof Pontén, and Søren M. Andersen

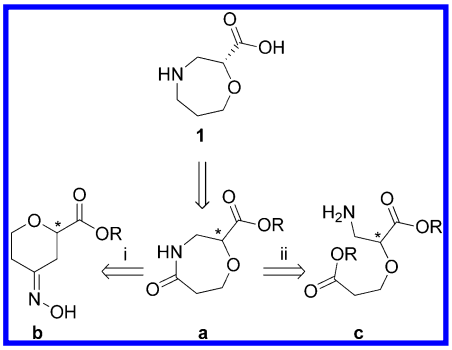
Innovative Medicines, Cardiovascular and Metabolic Diseases, Medicinal Chemistry, AstraZeneca R&D, Pepparedsleden 1, SE-43183 Mölndal, Sweden

ABSTRACT: A synthesis of *N*-Boc (2*R*)-1,4-oxazepane-2-carboxylic acid **1** has been developed in 39% yield over seven steps starting from methyl (2*R*)-glycidate **2**. The key step was a lipase-catalyzed regioselective lactamization of amino diester **5** into seven-membered lactam **6**. The transformation was performed using SpinChem rotating flow cell technology which simplified the work up and the recycling of the enzyme. Subsequent *N*-Boc protection followed by chemoselective borane reduction of the lactam moiety afforded 4-*tert*-butyl 2-methyl (2*R*)-1,4-oxazepane-2,4-dicarboxylate **8**. Finally, hydrolysis mediated by LiBr/Et_3N in wet acetonitrile yielded the title compound (2*R*)-4-(*tert*-butoxycarbonyl)-1,4-oxazepane-2-carboxylic acid **1**.

INTRODUCTION

Chiral 1,4-oxazepane-2-carboxylic acids are β-amino acid analogues and interesting scaffolds for drug development.[1] For a lead optimization program, we required hundreds of grams of enantiomerically pure *N*-Boc (2*R*)-1,4-oxazepane-2-carboxylic acid **1** (Scheme 1). We believed that this compound could be obtained via a selective reduction of lactam **a**. Such lactams have previously been synthesized from oxime **b** via a Beckmann rearrangement (transformation i).[2] However, since this transformation results in a mixture of regioisomers, we searched for other approaches to obtain the 5-oxo-oxazepane **a**. One route we found intriguing was the cyclization of a chiral amino diester **c**, also a potential precursor to the seven-membered lactam **a** (transformation ii). The cyclization could potentially also yield β-lactams and polymerization byproducts; however, we hoped to find conditions that would furnish **a** selectively.

Scheme 1. Retro synthetic routes



RESULTS AND DISCUSSION

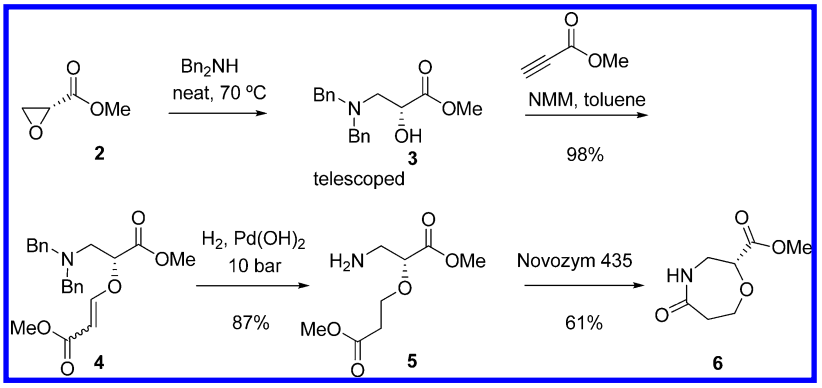
The chiral amino diester **5** was chosen for testing of the cyclization. This was prepared from methyl (2*R*)-glycidate **2** in a three-step sequence according to Scheme 2. On a 100 g scale, dibenzylamine was added to neat methyl (2*R*)-glycidate **2** at 70 °C, affording a quantitative yield of crude methyl (2*R*)-3-(dibenzylamino)-2-hydroxypropanoate **3**. Conjugate addition of **3** to methyl propiolate[3] gave a 93:7 mixture of the E and Z isomers of **4** in 98% overall yield and subsequently, hydrogenation using palladium hydroxide as a catalyst, afforded methyl (2*R*)-3-amino-2-(3-methoxy-3-oxopropoxy)propanoate **5** (82% w/w, 87% effective yield).[4] Upon neat storage we found that the amino diester **5** was unstable and slowly polymerized, but in a solution of, e.g., dioxane or methanol it was stable for weeks at +5 °C.

We attempted to cyclize diester **5** under a variety of conditions. At elevated temperature in xylenes, methanol or diglyme, no product was obtained. We also tried the cyclization in the presence of acid (benzenesulfonic acid), Lewis acid ($MgCl_2$), and base (K_2CO_3, MeONa), and again, unreacted starting material as the main component was obtained along with polymerization byproducts. We next turned our attention to an enzymatic intramolecular aminolysis. Amino esters have been reported to cyclize to lactams in the presence of crude pancreatic porcine lipase, although only a 10% conversion was observed after 1 week in the case of seven-membered lactams.[5] Recently, Stavila and Loos reported lipase-catalyzed aminolysis of a range of amino acids using Novozym 435, yielding the corresponding cyclic lactams including the seven-membered rings.[6] Lipase-catalyzed regioselective lactamization of amino diesters have to our knowledge not been reported, but this would offer a direct route to the 1,4-oxazepane-2-carboxylic acid scaffold. A screen of lipases[7] showed that Novozym 435 was able to catalyze the conversion of 2*R*-amino diester **5** into the corresponding seven-membered lactam **6**. Initially, the reaction was performed in standard batch mode. Without optimizing the process, we converted amino diester **5** to methyl (2*R*)-5-oxo-1,4-oxazepane-2-carboxylate **6** using 40% (w/w) Novozym 435 with 94% conversion within 26 h. After chromatography compound **6** was obtained in 61% yield. According to ^1H NMR analyses, various polymeric byproducts accounted for the remaining 39%. Since stirring of the

Received: May 26, 2014
Published: September 9, 2014

dx.doi.org/10.1021/op5001644 | *Org. Process Res. Dev.* 2014, 18, 1116−1119

Scheme 2. Synthesis of amino diester 5 and seven-membered lactam 6



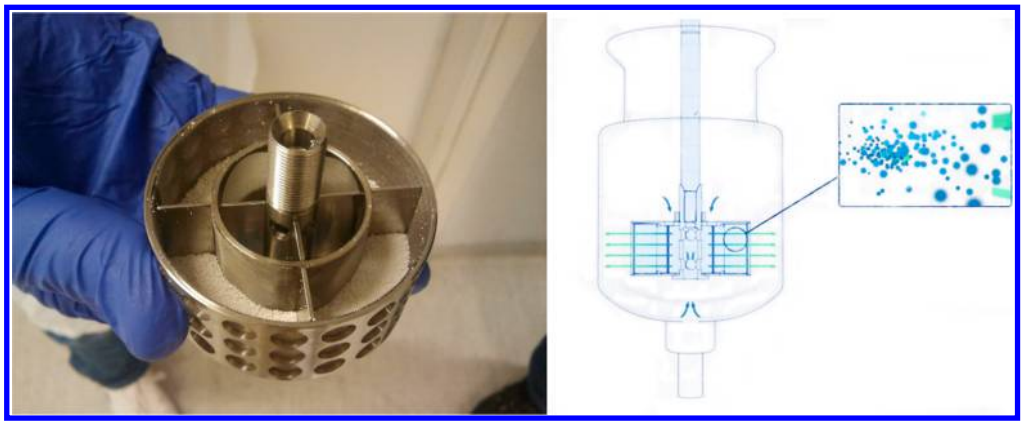


Figure 1. SpinChem rotating flow cell.

immobilized enzyme in standard batch mode generated fine particles causing blockage of the filter during workup, we decided to test a new rotating flow cell technology, SpinChem, which is a mechanical stirrer integrated with four compartments that can be charged with solids and resins (Figure 1).[8] When rotated, a flow goes into the top and bottom center of the rotating flow cell and out through the compartments packed with immobilized enzyme. In this way a turbulent flow is created in the reactor, and the generation of fine particles is minimized. In fact the rotating flow cell is self-filtrating as any large particles will be sequestered in the rotating disc.

With the SpinChem rotating flow cell, at the same scale and enzyme loading as in standard batch mode, a similar rate was observed (Figure 2). We found this new technology very versatile, since no filtration of immobilized enzyme was necessary after completion of reaction and it was very convenient to reuse the enzyme already packed in the disc. We ran a second round of cyclization with the rotating flow cell and obtained full conversion within 48 h.

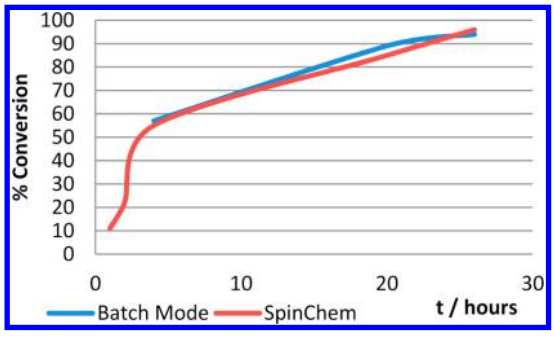
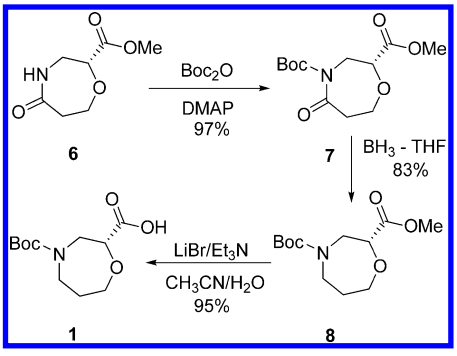


Figure 2. Rate comparison of transformations of **5** into **6** using batch mode and SpinChem rotating flow cell.

In order to obtain the carboxylic acid **1**, compound **6** was *N*-Boc protected followed by reduction using BH_3-DMS (Scheme 3).[9] The product, 4-*tert*-butyl (2*R*)-2-methyl 1,4-oxazepane-2,4-dicarboxylate **8**, was then hydrolyzed using LiOH in THF/water at room temperature to give the carboxylic acid **1**. However, since partial racemization was observed as well as generation of other impurities, we changed to the mild LiBr/NEt_3/wet acetonitrile hydrolysis method which afforded the carboxylic acid **1** in 95% assay yield with 99% ee.[10]

Scheme 3. Final steps to *N*-Boc protected (2*R*)-1,4-oxazepane-2-carboxylic acid 1



dx.doi.org/10.1021/op5001644 | Org. Process Res. Dev. 2014, 18, 1116−1119

CONCLUSION

We have developed a seven-step route to *N*-Boc (*R*)-1,4-oxazepane-2-carboxylic acid **1**, starting from readily available glycidate **2**. For the key cyclization of amine diester **5** into the seven-membered lactam **6**, we found that the immobilized lipase from *Candida antarctica*, Novozym 435, was an efficient catalyst ensuring 94% conversion within 26 h at 40% (w/w) loading. We tested the enzymatic transformation under flow-through conditions using SpinChem rotating flow cell, and although we did not observe any reaction rate enhancement, this technology facilitated the workup, and the enzyme could readily be reused.

EXPERIMENTAL SECTION

Commercially available solvents and reagents were used without purification. All reaction and operations were conducted under an inert nitrogen atmosphere, and unless otherwise noted, the temperature describes the mantle temperature of the reactor. LC analyses were performed using a Waters 600 instrument, C8 kromasil 100 column (50 × 4.6 mm, d_f = 5 μm), mobile phase 0.1 M NH_4OAc buffer/acetonitrile. HRMS analyses were performed using a Micromass LCT mass spectrometer. ^{1}H NMR measurements were performed on a Varian Mercury VX 400 spectrometer, operating at a ^{1}H frequency of 400 MHz or on Varian UNITY plus 400, 500, and 600 spectrometers, operating at ^{1}H frequencies of 400, 500, and 600 MHz, respectively. Chemical shifts are in ppm with the solvent as an internal standard. Coupling constants are in hertz. Purity is measured by % area in LC and wt % by Quantitative NMR. When wt % is noted, the yields have been corrected. Chiral purity was determined at 220 nm on a Chiralpak IA column using a 20% EtOH/DEA 100/0.5 in CO_2, 120 bar at 40 °C.

Methyl (2*R*)-3-(Dibenzylamino)-2-hydroxypropanoate (3). A reactor was charged with methyl (2*R*)-glycidate (**2**) (74 g, 97 wt %, 703 mmol) and dibenzylamine (128 g, 98 wt %, 633 mmol). The mixture was heated at 70 °C (reflux) for 48 h and then concentrated under reduced pressure to remove excess of methyl (2*R*)-glycidate to give a yellow thick oil of (**3**), which was used in the next step without any further purification (96 wt %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.78−2.89 (m, 2H), 3.49 (d, J = 13.6 Hz, 2H), 3.62 (s, 3H), 3.74 (d, J = 13.6 Hz, 2H), 4.21 (dd, J = 4.2 Hz, J = 6.8 Hz, 1H), 7.16−7.35 (m, 10H). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 52.2, 56.0, 58.7 (2C), 69.1, 127.2 (2C), 128.3 (4C), 129.0 (4C), 138.4 (2C), and 173.8. HRMS (ESI): m/z Calcd for $C_{18}H_{22}NO_3$: 300.1600 [M + H]; found 300.1590.

Methyl (2*E*/*Z*)3-{[(2*R*)-3-(Dibenzylamino)-1-methoxy-1-oxopropan-2-yl]oxy}acrylate (4). Toluene (120 mL) and 4-methylmorpholine (13.92 mL, 127 mmol) were added to the crude methyl (2*R*)-3-(dibenzylamino)-2-hydroxypropanoate (**3**) (633 mmol). The mantle temperature was set at 15 °C, and methyl propiolate (66.1 mL, 98 wt %, 728 mmol) was added during 30 min, keeping the reaction temperature below 30 °C. EXOTHERMIC! The reaction mixture was stirred at room temperature for 5 h and concentrated under reduced pressure to yield (**4**) as an oil (255.7 g, 93 wt %, 98% yield from (**2**)). The E/Z ratio was 100/8. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.9−3.02 (m, 2H), 3.53 (d, 2H), 3.64 (s, 3H), 3.66 (s, 2H), 3.70 (d, 2H), 4.41 (td, 1H), 4.86 (d, 0.08H), 5.20 (d, 1H), 6.33 (d, 0.08H), 7.16−7.34 (m, 11H), 7.43 (d, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) (only E-isomer) δ 51.13, 52.30, 54.64, 58.95, 79.19, 98.20, 127.14, 128.14, 128.22, 128.88, 128.98, 138.54, 161.01, 167.59, 169.04. HRMS (ESI): m/z Calcd for $C_{22}H_{26}NO_5$: 384.1811 [M + H]; found 384.1815.

Methyl (2*R*)-3-Amino-2-(3-methoxy-3-oxopropoxy)-propanoate (5). Moist 20% $Pd(OH)_2$ on charcoal (50% moisture, 18 g, 13 mmol) was dried under a stream of nitrogen until a 50% weight loss was reached and then suspended in methanol (200 mL) and added to a solution of methyl (2*R*)-3-((3-(dibenzylamino)-1-methoxy-1-oxopropan-2-yl)oxy)acrylate (**4**) (255.7 g, 93 wt %, 620 mmol) in methanol (1.8 L). The mixture was hydrogenated at 10 bar and 40 °C for 16 h. The hydrogenation vessel was flushed with nitrogen and the reaction mixture was filtered and concentrated under reduced pressure to an oil of (**5**) (135 g, 82 wt %, 87% yield). The product was dissolved in dioxane (2 L) and kept at +5 °C to avoid polymerization (51.85 g/L). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.4 (s, 2H), 2.55−2.73 (m, 2H), 2.90−2.97 (dd, J = 6.7, J = 13.5, 1H), 3.00−3.08 (dd, J = 3.8, J = 13.5, 1H), 3.69 (s, 3H) 3.72−3.74 (m, 1H), 3.75 (s, 3H), 3.87−3.98 (ddd, J = 3.7, J = 6.3, J = 13.5, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 34.7, 44.3, 51.6, 51.9, 66.1, 81.1, 171.6, 171.7. HRMS (ESI): m/z Calcd for $C_8H_{16}NO_5$: 206.1028 [M + H]; found 206.1018.

Methyl (2*R*)-5-Oxo-1,4-oxazepane-2-carboxylate (6), Batch Mode. Novozym 435 (10 g) was added to a solution of amino diester (**5**) in dioxane (460 mL, 23.9 g 5, 116 mmol) and the slurry was stirred at 40 °C. The conversion was followed by proton NMR: t = 1 h: 30%, t = 4 h: 57%, t = 20 h: 89% (crude product starts crystallizing), t = 26 h: 94%. The immobilized enzyme was filtered off, and the filtrate was concentrated under reduced pressure to give an oil of (**6**), which solidified (28.8 g). The crude product was slurried in a solution of MTBE (2 vol) and 2-propanol (1 vol), filtered off, and dried prior to SFC chromatography (Kromasil Sil 10 μm, 10% isopropanol in CO_2). Yield: 12.4 g, 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.63 (ddt, 1H, J = 1.4, J = 1.4, J = 6.6, J = 15.7), 2.83 (ddd, 1H, J = 2.3, J = 9.7, J = 15.7), 3.59 (m, 2H), 3.69 (m, 1H,), 3.77 (s, 3H), 4.19 (m, 2H), 6.98 (s, 1H). ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 39.99, 45.76, 52.55, 64.27, 78.72, 169.14, 177.08. Chiral purity: 99% ee. HRMS (ESI): m/z Calcd for $C_7H_{14}NO_4$ 174.0766 [M + H]; found 174.0760.

Methyl (2*R*)-5-Oxo-1,4-oxazepane-2-carboxylate (6), in SpinChem. The rotating flow cell integrated in the stirrer to the reactor was charged with Novozym 435 (10 g), and the solution of amino diester (**5**) in dioxane (460 mL, 23.9 g, 116 mmol) was added to the reactor. The reaction solution was stirred at 40 °C, creating a turbulent flow through the enzyme holder. The conversion was followed by proton NMR: t = 1 h: 11%, t = 2 h: 22%, t = 4 h: 55%, t = 20 h: 85% (the crude product crystallizes at this point), t = 26 h: 96%. The reaction solution was worked up as in the batch mode, and 12.2 g (60%) was obtained. After drainage of the product solution another portion of 460 mL of substrate in dioxane (25 g of aminoester) was added to the reactor, and a second enzymatic transformation was carried out. Complete conversion was completed after 48 h.

4-*tert*-Butyl 2-methyl (2*R*)-5-oxo-1,4-oxazepane-2,4-dicarboxylate (7). Di-*tert*-butyl dicarbonate (17.6 g, 79.05 mmol) was added to a suspension of methyl (2*R*)-5-oxo-1,4-oxazepane-2-carboxylate (**6**) (13.4 g, 77.5 mmol), DMAP (0.189 g, 1.55 mmol), and THF (120 mL). The mixture was stirred at 20 °C for 2 days and concentrated to dryness. Traces of residual *tert*-BuOH were azeotropically removed using THF (2 × 50 mL). A pale yellow oil of (**7**) was obtained (22.6 g, 91

dx.doi.org/10.1021/op5001644 | *Org. Process Res. Dev.* 2014, 18, 1116−1119

wt %, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H); 2.77 (ddd, 1H, J = 16.1, J = 7.0, J = 1.9 Hz); 2.94 (ddd, 1H, J = 16.1, J = 9.3, J = 2.5 Hz); 3.75 (s, 3H); 3.80 (ddd, 1H, J = 12.9, J = 9.1, J = 2.0 H); 3.91 (dd, 1H, J = 16.0, J = 7.2 Hz); 4.12−4.30 (m, 2H); 4.38 (dd, 1H, J = 16.0, J = 1.4 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ 27.9, 42.3, 48.8, 52.6, 63.4, 77.5, 83.8, 152.1, 169.0, 172.6. HRMS (ESI): m/z Calcd for $C_{12}H_{19}NO_6Na$ 296.1111 [M + Na]; found 296.1121.

4-*tert*-butyl-2-methyl (2R)-1,4-oxazepane-2,4-dicarboxylate (8). BH_3-DMS (11.40 g, 150.1 mmol) was added during 10 min to a solution of 4-*tert*-butyl 2-methyl (2*R*)-5-oxo-1,4-oxazepane-2,4-dicarboxylate (**7**) (22.54 g, 91 wt %, 75.1 mmol) in THF (150 mL) (slightly exothermic), and the resulting homogeneous mixture was allowed to stir at 20 °C overnight. The mixture was added to MeOH (150 mL), stirred at room temperature for 1 h and concentrated under reduced pressure. Residual $(MeO)_3B$ was azeotropically removed with MeOH (2 × 100 mL) and the product (**8**) was obtained as a colorless oil (19.6 g, 82 wt %, 83% yield). ^{1}H NMR (400 MHz, MeOD, 1:1 mixture of rotamers) δ 1.51 (s, 9H); 1.84−1.93 (m, 2H); 3.20−3.34 (m, 1H); 3.42−3.56 (m, 1H); 3.70−3.81 (m, 5H); 4.02−4.12 (m, 2H); 4.36−4.41 (m, 1H). ^{13}C NMR (100.6 MHz, MeOD, 1:1 mixture of rotamers) δ 28.6, 31.0, 31.5, 47.8, 48.2, 51.0, 51.2, 52.6, 68.6, 68.7, 77.6, 77.8, 81.4, 81.6, 156.7, 156.9, 172.8, 172.9. HRMS (ESI): m/z Calcd for $C_{12}H_{21}NO_5Na$ 282.1318 [M + Na]; found 282.1319.

(2R)-4-(tert-butyloxycarbonyl)-1,4-oxazepane-2-carboxylic acid (1). Triethylamine (18.6 g, 184 mmol) and LiBr (26.6 g, 307 mmol) were added to a solution of (*R*)-4-*tert*-butyl 2-methyl 1,4-oxazepane-2,4-dicarboxylate (**7**) (19.4 g, 82% w/w, 61.4 mmol) in acetonitrile (100 mL) and water (2 mL) at 0 °C, and the reaction mixture was stirred at 20 °C overnight. Most of the solvent was removed under reduced pressure and MTBE (100 mL) and water (150 mL) added. The layers were separated and EtOAc (100 mL) added. 2 M $KHSO_4$ was added until pH 2, and the aqueous phase was extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with water (30 mL) and concentrated under reduced pressure affording (**1**) as a crude solid (15.4 g, 93 wt %, 95% yield). The solid was dissolved in refluxing MTBE (30 mL). Upon addition of heptane (50 mL) a slightly turbid solution was obtained. The mixture was allowed to attain room temperature and stirred overnight. The solids were filtered off and washed with 30% MTBE in heptane (20 mL) to yield (**1**) as colorless crystals (11.6 g, 99 wt %, >99% ee). $[\alpha]_D^{20}$ +1 (c = 1, CH_3CN). ^{1}H NMR (400 MHz, MeOD, mixture of rotamers) δ 1.46 (s, 9H); 1.77−1.90 (m, 2H); 3.15−3.77 (m, 4H); 3.91−4.17 (m, 2H); 4.22−4.32 (m, 1H). ^{13}C NMR (100.6 MHz, MeOD, mixture of 2 rotamers) δ 28.5, 28.6, 31.0, 31.3, 47.8, 47.9, 51.4, 68.6, 69.0, 77.7, 78.0, 81.4, 81.7, 156.8, 157.0, 174.0, 174.2. HRMS (ESI): m/z Calcd for $C_{11}H_{19}NO_5Na$ 268.1161 [M + Na]; found 268.1157.

AUTHOR INFORMATION

Corresponding Author

*E-mail: carl-johan.aurell@astrazeneca.com.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) See review: Miller, J. A.; Nguyen, S. T. *Mini-Rev. Org. Chem.* **2005**, *2*, 39−45.

(2) Dowle, M. D.; Gore, P. M.; Hodgson, S. T.; Johnson, M. R.; Judd, D. B.; Redfern, T. J.; Robinson, J. E.; Swanson, S.; Trivedi, N. World Patent WO2003097618, 2003.

(3) (a) Hiramatsu, N.; Takahashi, N.; Noyori, R.; Mori, Y. *Tetrahedron* **2005**, *61*, 8589−8597. (b) Wang, J.; Wang, H.; Ren, H. *Synth. Commun.* **2010**, *40*, 980−983.

(4) At two occations we encountered problems using $Pd(OH)_2$. This could be due to batch variations from different suppliers. Dry palladium(0) on charcoal also works well with methanol in this reduction.

(5) Gutman, A. L.; Meyer, E.; Yue, X.; Abell, C. *Tetrahedron Lett.* **1992**, *33*, 3943−3946.

(6) Stavila, E.; Loos, K. *Tetrahedron Lett.* **2013**, *54*, 370−372.

(7) The following enzymes were screened: (a)*Candida Antarctica* Lipase B (Novozym 435); (b) *Porcine Pancreas* type II, crude Lipase; (c) *Candida Antarctica* Lipase A CLEA; (d) *Aspergillus spp* (protease), Acylase I; (e) *Rhizomucor oryzae* (Lipozyme RM IM); (f) *Candida Cylindracea* type VII, Lipase; (g) *Pseudomonas fluorescens* (Lipase AK Amano); (h) *Rhizopus oryzae* (Lipase F-AP15); (i) *Peniciium camemberti* (Lipase G Amano 50); (j)*Candida rugosa* (Lipase AYS Amano); (k) *Aspergillus niger* (Lipase AS Amano); (l) *Mucor javanicus* (Lipase M Amano 10).

(8) SpinChem is supplied by Nordic Chem Quest AB, Tvistevägen 48, SE-90736 Umeå, Sweden. http://www.spinchem.com. The rotating flow cell technology has been proof tested in reactors up to 800 L.

(9) Early introduction of the Boc group was done to avoid the strongly coordinated boron species complexation with the amine moiety which made the work up difficult.

(10) Mattsson, S.; Dahlström, M.; Karlsson, S. *Tetrahedron Lett.* **2007**, *48*, 2497−2499.