Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. *85* (1) 1–8 (2020)
JSCS–5278

UDC 547.584+547.574.2–326+542.913:576+615.9:57–188
Original scientific paper

Synthesis of novel phthalimido oxime pseudoesters and evaluation of their cytotoxicity

ASMA MEHREZ[1], IBTISSEM CHAKROUN[2], DALILA MTAT[1], HÉDI BEN MANSOUR[3] and RIDHA TOUATI[1]*

[1]*Laboratory of Asymmetric Organic Synthesis and Homogeneous Catalysis (UR11ES56), Faculty of Sciences of Monastir, Avenue of the Environment, 5019 Monastir, Tunisia,* [2]*Laboratory for Analysis, Treatment and Recovery of Environmental Pollutants and Products, Faculty of Pharmacy, Avicenne Street, 5000 Monastir, Tunisia and* [3]*Research Unit Analyzes and Processes Applied to the Environment (UR17ES32), Higher Institute of Applied Sciences and Technology of Mahdia, 5111 Mahdia, Tunisia*

(Received 2 March 2018, revised 16 July 2019, accepted 1 August 2019)

Abstract: A series of novel optically pure oxime pseudoesters derivatives were synthesized by the reaction of substitute keto oximes with various *N*-substituted *α*-amino acids chlorides in the presence of triethylamine and dichloromethane at 0 °C, and their structures were characterized by IR and 1D-NMR methods. The synthesized compounds were tested for their ability to inhibit the proliferation of human colon cancer cells and human epithelial cells. Some of them were revealed to have a significant cytotoxic effect.

Keywords: oxime esters; *α*-amino acids; stereoselective; cytotoxic; biological activity; enantiomer.

INTRODUCTION

Cancer remains a serious human health problem despite considerable progress in the understanding of its biology and pharmacology. The main problem is that cancer is not one disease, but a group of diseases affecting different organs and systems of the body.

Cancer develops due to abnormal and uncontrolled cell division, frequently at a rate greater than that of most normal body cells.[1]

For some types of disseminated cancers, chemotherapy is the only effective therapy because it distributes anticancer drugs through the circulatory system. Oxime pseudoesters are a small, but important, class of biologically useful compounds for the synthesis of fragrances,[2] crop protection, and therapeutic studies.[3] They are useful building blocks in peptide synthesis.[4] Oxime pseudoesters are

* Corresponding author. E-mail: ridhato@yahoo.fr
https://doi.org/10.2298/JSC180302082M

Available on line at www.shd.org.rs/JSCS/

(CC) 2019 SCS.

selective covalent inhibitors of serine hydrolase retinoblastoma-binding protein 9 (RBBP9) and cleave DNA under photolytic conditions.[5,6] They also possess fungicidal,[7] herbicidal,[8] insecticidal and antitumor activity.[9] Oxime pseudoesters of dihydrocoumaric acid have been synthesized and they were reported to have antibacterial activity.[10] Aromatic benzophenone oxime esters and dibenzosuberone oxime esters are pharmacologically important.[11] Vanillin derived piperidin-4-one oxime esters have been tested for their antioxidant and antimicrobial potential.[12] The oxime pseudoesters derived from nafimidone have been tested as potential anticonvulsant compounds.[13]

Several methods have been developed for the preparation of oxime pseudoesters derivatives.[3] The most common method is the condensation of acid chlorides with oximes under basic conditions or the use of acid anhydrides in presence of strong acids.[2,6,10,12,13]

Oxime pseudoesters can be prepared using *α*,*β*-unsaturated aldehydes and oximes employing a *N*-heterocyclic carbene as a redox esterification catalyst,[14] or by treatment of alkyl- or aryl-substituted oximes with aliphatic or aromatic acids in the presence of the *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDCI) reagent.[15]

A large number of studies on their synthesis and biological activities have been reported during the last thirty years.[16] However, no attention was paid to the stereoselective synthesis of chiral oxime pseudoesters derivatives. Hence, in continuation of our research aimed at the preparation of natural and non-natural compounds of biomedical importance,[17] and in connection with ongoing investigations on the reactivity of natural amino acids,[18] herein, an efficient and easy methodology is reported for the synthesis of a series of new optically pure oxime pseudoesters **5a**–**k** starting from the commercially available acetophenone derivatives **1a**–**f** and natural amino acids **3a**–**d**, which are of considerable interest as chiral pool agents since they are easily accessible and inexpensive enantiomerically pure compounds.

EXPERIMENTAL

Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Silica gel (100–200 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX spectrometer. ^{1}H-NMR and ^{13}C--NMR spectral data were recorded on Advance Bruker 300 spectrometer (300 MHz) with $CDCl_3$ as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were obtained by Agilent 5973- network mass selective detector (EI).

*General procedure for the preparation of oximes **2a**–**f***

Acidic hydroxylamine ($NH_2OH{\cdot}HCl$, 0.1 mol) was added dropwise to a stirred solution of substituted acetophenone (0.02 mol) in 95 % EtOH (150 mL) and pyridine (8 mL, 0.1 mol)

(CC) 2019 SCS.

at room temperature. The resulting mixture was refluxed for 0.5–2 h (until the starting material was completely consumed as indicated by TLC), and cooled in cold water for1 h. The precipitate was collected by suction, washed with warm water (3×50 mL) and dried in a vacuum oven. The crude compound was recrystallized in ethanol to give a white solid.

Synthesis of N-*phthaloyl-L-amino acids* ***4a–d***

A solution of the required amino acid (1 equiv.) in toluene was added to a solution of phthalic anhydride (1 equiv.) and triethylamine (1.2 equiv.). The reaction mixture was then refluxed on a water bath for about 4–5 h. After completion of the reaction, the resulting solution was separated. The organic phase was washed with water until neutral, dried over $MgSO_4$ and filtered. The filtrate was evaporated and the residue purified by column chromatography on silica gel to give the required compounds (**4a**–**d**).

Synthesis of oxime pseudoesters ***5a–k***

A mixture of an *N*-phthaloyl-L-amino acid **4a**–**d** (1 equiv.) and thionyl chloride (2 mL, slow addition) were mixed together and the contents were heated at 55 °C for 4 h. The reaction mixture was cooled to room temperature and kept in an ice bath. Then, a solution of oxime **2** (1 equiv.), triethylamine (1.2 equiv.) in dichloromethane (CH_2Cl_2, 30 mL) was added subsequently to the reaction mixture and the contents were stirred at room temperature for 2 h. On completion of the reaction, checked by thin-layer chromatography (TLC) analysis, the solvent was removed under reduced pressure and extracted with dichloromethane (3×30 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The thus obtained residue was purified by silica gel chromatography (hexane/ethyl acetate) to afford the desired oxime pseudoesters derivatives (**5a**–**k**).

Cell lines and culture medium

The human colon carcinoma cells (Caco-2; ECACC 86010202) and the epidermoid carcinoma epithelial cells (Hep-2; ATCC CCL-23) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % of fetal bovine serum, 1 % non-essential amino acids and 1 % penicillin/streptomycin (Invitrogen). At 85–90 % confluence, the cells were harvested using a 0.25 % trypsin/EDTA solution and sub-cultured onto 96-well plates according to the experimental requirements.

Cytotoxicity screening assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay described earlier[19] was used to screen the cytotoxic activity of the isolated compound. Briefly, the Caco-2 and Hep-2 cell lines (1×10^5 cells/well) were grown overnight on 96-well flat bottom cell culture plates, incubated 24 h. When a partial monolayer had formed, the supernatant was flicked off, the monolayer washed once with medium and 100 μL of different concentrations (10, 5, 2.5 and 1.25 mg mL^{-1}) of pure compound were added to the cells.in the microtitre plates. After 24 h, the cells were washed and treated with 0.01 mL MTT reagent (Invtrogen) prepared in 5.0 mg mL^{-1} phosphate buffered saline (PBS) per well. The plates were incubated at 37 °C in a 5 % CO_2 atmosphere for 4 h, and 0.1 mL dimethyl sulfoxide (DMSO) was added. After overnight incubation at 37 °C, the absorbance was measured at 550 nm using an ELISA reader (Thermo Scientific Multiskan FC) and was compared with the control cultures without compound. The results were generated from 3 independent experiments and each experiment was performed in triplicate. The percentage growth inhibition was calculated using following formula:

$$\text{Cell inhibition, \%} = 100–100\{(A_t–A_b)/(A_c–A_b)\}$$

(CC) 2019 SCS.

where, A_t = absorbance value of the test compound, A_b = absorbance value of the blank and A_c = absorbance value of the control.

Stock solutions (5 mg/mL) of pure compounds were prepared in dimethyl sulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25 %. Serial dilutions with culture media were prepared just prior to the test.

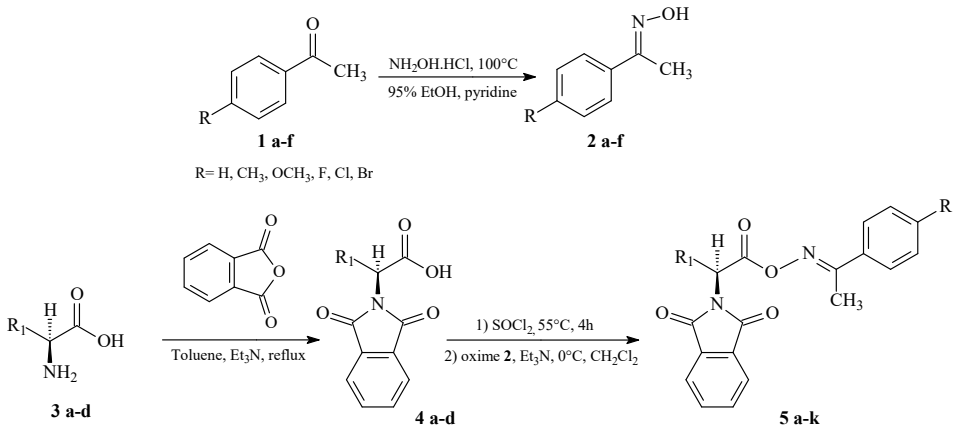
Statistical analysis

The results are expressed as mean ± *SEM*. The data were statistically analyzed by one-way analysis of variance (ANOVA) to determine differences among the groups and the Tukey test as a post-hoc. All the statistical analysis was conducted using Statistical Package for Social Science (SPSS for Windows; v. 19.0, USA) and differences were considered statistically significant at $p < 0.05$.

Analytical and spectral data of the synthesized compounds are given in the Supplementary material to this paper.

RESULTS AND DISCUSSION

The substituted oximes **2a**–**f** were synthesized according to the literature procedure[6b,20] shown in Scheme 1. The condensation of acetophenone derivatives **1a**–**f** with hydroxylamine hydrochloride and pyridine gave white shiny coloured oximes **2a**–**f** in 90 % yield.



5a: R= CH_3, R_1= CH_3 **5e**: R= Br, R_1= CH_3 **5i**: R= H, R_1= $CH_2C_6H_3(OH)_2$
5b: R= OCH_3, R_1= CH_3 **5f**: R= CH_3 , R_1= $CH_2CH(CH_3)_2$ **5j**: R= OCH_3, R_1= $CH_2C_6H_3(OH)_2$
5c: R= F, R_1= CH_3 **5g**: R= Cl, R_1= $CH_2CH(CH_3)_2$ **5k**: R= Cl, R_1= $CH_2C_6H_3(OH)_2$
5d: R= Cl, R_1= CH_3 **5h**: R= CH_3, R_1= $CH(CH_3)CH_2CH_3$

Scheme 1. Synthesis of oxime pseudoesters **5a**–**k**.

The *α*-*N*-phthalimido amino acids (compounds **4a**–**d**) were synthesized according to a method described in the literature[18a] by allowing phthalic anhydride to react with a number of commercially available amino acids in refluxing apolar solvents, such as toluene, in the presence of triethylamine and separation of the formed water (Scheme 1).

(CC) 2019 SCS.

Treatment of **4a**–**d** with thionyl chloride followed by treatment with keto oxime in anhydrous dichloromethane in the presence of Et_3N at 0 °C to room temperature provided the corresponding *N*-substituted phthaloyl derivatives **5a**–**k** with an average yield of 76 % in two steps and after purification. Their structures were established by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry.

The antiproliferative potential of the synthesized compounds **5a**–**d**, **5f** and **5h**–**j** was determined *in vitro* against two cancer cell lines, *i.e.*, Hep-2 and Caco-2. The cytotoxicity values were obtained as inhibition of different concentrations and the results are summarized in Tables I and II.

TABLE I. Cytotoxic activity of some derivatives against Caco-2 cells; the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay described earlier[19] was used to screen the cytotoxic activity of the isolated compound

Compound	Concentration, mg mL^{-1}			
	10	5	2.5	1.25
5a	< 20 %	< 20 %	< 20 %	< 20 %
5b	59.67±1.91	40.98±1.22	22.56±2.03	25.17±1.15
5c	52.94±1.22	43.01±2.16	41.37±0.9	35.01±0.85
5d	56.19±0.78	44.41±1.08	37.96±1.2	29.06±0.63
5f	< 20 %	< 20 %	< 20 %	< 20 %
5h	< 20 %	< 20 %	< 20 %	< 20 %
5i	41.03±3.54	40.15±1.56	28.92±1.94	14.02±0.76
5j	< 20 %	< 20 %	< 20 %	< 20 %

TABLE II. Cytotoxic activity of some derivatives against Hep-2 cells

Compound	Concentration, mg mL^{-1}			
	10	5	2.5	1.25
5a	< 20 %	< 20 %	< 20 %	< 20 %
5b	48.22±1.12	20.15±0.86	11.02±1.61	7.89±0.74
5c	57.85±2.13	43.21±1.92	21.11±0.83	27.02±2.35
5d	61.02±1.69	56.12±2.01	31.12±0.77	10.25±0.92
5f	< 20 %	< 20 %	< 20 %	< 20 %
5h	< 20 %	< 20 %	< 20 %	< 20 %
5i	53.91±1.6	50.77±2.51	17.01±1.38	9.49±1.02
5j	< 20 %	< 20 %	< 20 %	< 20 %

The results demonstrated that only four compounds, **5b**–**d** and **5i**, have moderate potency of around 40 % inhibition at 10 mg mL^{-1} against Hep-2 and Caco-2, while the other compounds, **5a**, **5f**, **5h** and **5j** were inactive against the two cancer cell lines (inhibition effect < 20 %). It was observed that when the methoxy group was attached at the *para* position of the phenyl ring (compound **5b**), the activity reduced to 48.22 % against the Hep-2 cell line. Replacing the substituent at the *para* position by an electron withdrawing group caused a decrease in the anticancer activity as compared to compound **5b** against the Caco-2

(CC) 2019 SCS.

cell line. This could be justified by the fact that compounds bearing electron withdrawing groups, such as fluoro (**5c**) and chloro (**5d**) substituents at the *para* position of the phenyl ring exhibited activity at inhibitory ratios values 52.94 and 56.19 %, respectively, against the Caco-2 cell line and 57.85 and 61.02 %, respectively, against the Hep-2 cell line.

Compound **5i** also showed activity against Hep-2 at 53.91 and 41.03 % against Caco-2 cell lines. It could be concluded that the cytotoxicities of the resulting oxime pseudoesters derivatives are significantly correlated with the nature of the substituent group.

CONCLUSIONS

In summary, a new series of optically pure phthalimido oxime pseudoesters derivatives were prepared and their cytotoxic activities against two human cancer cells lines, Caco-2 and Hep-2, evaluated. Some derivatives exhibited strong cytotoxic activity and therefore, further structural modifications and *in vivo* anti-tumor activity studies are to be undertaken. The present findings could provide new evidence showing the relationship between chemical structure and biological activity and may be useful for the design of novel chemotherapeutic drugs.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically at the pages of journal website: http://www.shd-pub.org.rs/index.php/JSCS, or from the corresponding author on request.

ИЗВОД

СИНТЕЗА НОВИХ ПСЕУДОЕСТАРА ФТАЛИМИДО ОКСИМА И ПРОЦЕНА ЊИХОВЕ ЦИТОТОКСИЧНОСТИ

ASMA MEHREZ[1], IBTISSEM CHAKROUN[2], DALILA MTAT[1], HÉDI BEN MANSOUR[3] и RIDHA TOUATI[1]

[1]Laboratory of Asymmetric Organic Synthesis and Homogeneous Catalysis (UR11ES56), Faculty of Sciences of Monastir, Avenue of the Environment, 5019 Monastir, Tunisia, [2]Laboratory for analysis, treatment and recovery of environmental pollutants and products, Faculty of Pharmacy, Avicenne Street, 5000 Monastir, Tunisia и [3]Research Unit Analyses and Processes Applied to the Environment (UR17ES32), Higher Institute of Applied Sciences and Technology of Mahdia, 5111 Mahdia, Tunisia

Синтетисана је серија нових, оптички чистих деривата псеудоестара оксима, реакцијом супституисаних кето-оксима са различитим хлоридима *N*-супституисаних *α*-амино киселина, у присуству триетил-амина, у дихлорметану на 0 °C. Њихове структуре су утврђене IR и 1D-NMR спектроскопским методама. Испитана је активност синтетисаних једињења према инхибицији пролиферације хуманог канцера дебелог црева и хуманих епителних ћелија. Нека од испитиваних једињења показују значајан цитотоксичан ефекат.

(Примљено 2. марта 2018, ревидирано 16 јула 2019, прихваћено 1. августа 2019)

REFERENCES

1. S. P. Gupta, *Chem. Rev*. **94** (1994) 1507 (https://doi.org/10.1021/cr00030a003)
2. a) N. A. Zhukovskaya, E. A. Dikusar, V. I. Potkin, O. G. Vyglazov, *Chem. Nat. Compd.* **45** (2009) 148 (http://dx.doi.org/10.1007/s10600-009-9264-7); b) E. A. Dikusar, N. A.

(CC) 2019 SCS.

Zhukovskya, *Russ. J. Org. Chem.* **44** (2008) 1389 (http://dx.doi.org/10.1134/S1070428008090248)
3. G. V. Crichlow, K. F. Cheng, D. Dabideen, M. Ochani, B. Aljabari, V. A. Pavlov, E. J. Miller, E. Lolis, Y. Al-Abed, *J. Biol. Chem.* **282** (2007) 3089 (http://dx.doi.org/10.1074/jbc.M701825200)
4. I. Hayashi, K. Shimizu, *Bull. Chem. Soc. Jpn.* **56** (1983) 3197 (http://dx.doi.org/10.1248/cpb.17.1937)
5. D. A. Bachovchin, M. R. Wolfe, K. Masuda, S. J. Brown, T. P. Spicer, V. Fernandez--Vega, P. Chase, P. S. Hodder, H. Rosen, B. F. Cravatt, *Med. Chem. Lett.* **20** (2010) 2254 (http://dx.doi.org/10.1016/j.bmcl.2010.02.011)
6. a) J. R. Hwu, S. Tsay, S. C. Hong, M. Hsu, C. Liu, S. P. Chou, *Bioconjugate Chem.* **24** (2013) 1778 (http://dx.doi.org/10.1021/bc400060h); b) P. J. Bindu, K. M. Mahadevan, N. D. Satyanarayan, T. R. Ravikumar Naik, *Bioorg. Med. Chem. Lett.* **22** (2012) 898 (http://dx.doi.org/10.1016/j.bmcl.2011.12.037)
7. X. H. Liu, L. P. Zhi, B. A. Song, H. L. Xu, *Chem. Res. Chin. Univ.* **24** (2008) 454 (http://dx.doi.org/10.1016/S1005-9040(08)60095-1)
8. T. G. Li, J. P. Liu, J. T. Han, B. Fu, D. Q. Wang, M. G. Wang, *Chin. J. Org. Chem.* **29** (2009) 898 (http://dx.doi.org/10.18596/jotcsa.290589)
9. B. A. Song, X. H. Liu, S. Yang, D. Y. Hu, L. H. Jin, Y. T. Zhang, *Chin. J. Org. Chem*. **25** (2005) 507 (http://dx.doi.org/10.1584/jpestics.D13-001)
10. Y. Gao, J. Song, S. Shang, D. Wang, J. Li, *BioResources* **7** (2012) 4150 (http://dx.doi.org/10.2174/157018013804725071)
11. B. B. Wylie, E. I. Isaacson, G. N. Delgado, *J. Pharm. Sci.* **54** (1965) 1373 (http://dx.doi.org/10.1002/jps.2600540932)
12. S. T. Harini, H. Vijay Kumar, J. Rangaswamy, N. Naik, *Bioorg. Med. Chem. Lett.* **22** (2012) 7588 (http://dx.doi.org/10.1016/j.bmcl.2012.10.019)
13. A. Karakurt, M. A. Alagöz, B. Sayoğlu, Ü. Çaliş, S. Dalkara, *Eur. J. Med. Chem.* **57** (2012) 275 (http://dx.doi.org/10.1016/j.ejmech.2012.08.037)
14. D. Enders, A. Grossmann, D. V. Craen, *Org. Biomol. Chem.* **11** (2013) 138 (http://dx.doi.org/10.1039/c2ob26974k)
15. S. C. Santosh Kumar, N. V. Kumar, P. Srinivas, B. K. Bettadaiah, *Synthesis* **46** (2014) 1847 (http://dx.doi.org/10.1055/s-0034-1378350)
16. R. Q. Huang, H. Y. Li, J. A. Ma, D.W. Qiu, *Chem. J. Chin. Univ.* **17** (1996) 571 (http://dx.doi.org/10.1002/jhet.5570430409)
17. a) C. Mordant, C. Cano de Andrade, R. Touati, V. Ratovelomanana-Vidal, B. Ben Hassine, J. P. Genêt, *Synthesis* **15** (2003) 2405 (http://dx.doi.org/10.1055/s-2003-42397); b) R. Touati, V. Ratovelomanana-Vidal, B. Ben Hassine, J. P. Genêt, *Tetrahedron: Asymmetry* **17** (2006) 3400 (http://dx.doi.org/10.1016/j.tetasy.2006.11.048); c) C. Mordant, S. Reymond, H. Tone, D. Lavergne, R. Touati, B. Ben Hassine, V. Ratovelomanana-Vidal, J. P. Genêt, *Tetrahedron* **63** (2007) 6115 (http://dx.doi.org/10.1016/j.tet.2007.03.036); d) S. Prévost, S. Gauthier, M. C. de Andrade, C. Mordant, R. Touati, P. Lesot, P. Savignac, T. Ayad, P. Phansavath, V. Vidal, J. P. Genêt, *Tetrahedron: Asymmetry* **21** (2010) 1436 (http://dx.doi.org/10.1016/j.tetast.2010.05.017); e) M. Perez, P. G. Echeverria, E. Martinez-Arripe, M. Ez Zoubir, R. Touati, Z. Zhang, J. P. Genêt, P. Phansavath, T. Ayad, V. Ratovelomanana-Vidal, *Eur. J. Org. Chem.* **27** (2015) 5949 (http://dx.doi.org/10.1002/ejoc.201500661)

(CC) 2019 SCS.

18. a) A. Essersi, R. Touati, B. Ben Hassine, *Lett. Org. Chem.* **7** (2010) 69 (http://dx.doi.org/10.2174/157017810790533869); b) A. Ould Aliyenne, J. Kraiiem, Y. Kacem, B. Ben Hassine, *C. R. Chim.* **10** (2007) 251 (http://dx.doi.org/10.13140/RG.2.1.1067.0247); c) Y. Kacem, A. Bouraoui, V. Ratovelomanana-Vidal, J. P. Genêt, B. Ben Hassine, *C. R. Chim.* **5** (2002) 611 (http://dx.doi.org/S1631-0748(02)01418-2)
19. M. Krifa, A. Della, I. Bouhlel, J. Robert, A. Cherif, D. Barillier, R. Mosrati, L. Chekir-Ghedira, H. Ben Mansour, *Environ Sci. Pollut. Res*. **20** (2013) 3842 (http://dx.doi.org/10.1007/s11356-012-1322-1)
20. W. W. Tan, B. Wu, Y. Wei, N. Yoshikai, *Org. Synth.* **95** (2018) 1 (https://doi.org/10.15227/orgsyn.95.0001).

(CC) 2019 SCS.