Journal of Fluorine Chemistry 131 (2010) 217–220

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

An optimized protocol for the multigram synthesis of 3-(trifluoromethyl)bicyclo[1.1.1]pent-1-ylglycine (CF_3-Bpg)

Pavel K. Mykhailiuk [a,b,*], Nataliia M. Voievoda [a,b], Sergii Afonin [c], Anne S. Ulrich [c,d], Igor V. Komarov [a,b]

[a] Department of Chemistry, Kyiv National Taras Shevchenko University, Vul. Volodymyrska 64, 01033 Kyiv, Ukraine
[b] Enamine Ltd., Vul. Oleksandra Matrosova 23, 01103 Kyiv, Ukraine
[c] Karlsruhe Institute of Technology (KIT), Forschungszentrum Karlsruhe, Institut für Biologische Grenzflächen, POB 3640, 76021 Karlsruhe, Germany
[d] KIT, Universität Karlsruhe, Institut für Organische Chemie, Fritz-Haber-Weg 6, 76133 Karlsruhe, Germany

ARTICLE INFO

Article history:
Received 25 July 2009
Received in revised form 6 October 2009
Accepted 7 October 2009
Available online 15 October 2009

Keywords:
Fluorine
Amino acids
Conformational rigidity
Large-scale synthesis

ABSTRACT

An optimized procedure for the multigram synthesis of 3-(trifluoromethyl)bicyclo[1.1.1]pent-1-ylglycine (CF_3-Bpg) has been developed. The overall yield of the synthesis for the optimized up-scaling was increased from 35% to 53%. Moreover, conditions for separating the key isomeric aminonitriles **7** and **8** by crystallization were found, which greatly facilitated the isolation of **8** on a multigram scale. Following this optimized protocol, 100 g of optically pure CF_3-Bpg have been synthesized.

1. Introduction

In 2006 we reported the synthesis of 3-(trifluoromethyl)bicyclo[1.1.1]pent-1-ylglycine (**1**, CF_3-Bpg) (Fig. 1) [1]. This CF_3-substituted conformationally rigid α-amino acid was prepared as a specific label to study membrane-bound peptides by solid-state ^{19}F NMR. Having been designed as an analogue of non-polar aliphatic amino acids, it can be readily used to replace naturally occurring residues of leucine, isoleucine, valine, alanine, and methionine, which are highly abundant in membrane proteins and peptides. The applicability of CF_3-Bpg as a ^{19}F NMR label was evaluated by comparison with the established reporter 4-CF_3-phenylglycine (CF_3-Phg) [2–4], and the structural compatibility of both amino acids was proven [5,6]. CF_3-Bpg was successfully applied thereafter to elucidate the conformation, membrane alignment, and the dynamic behaviour of several natural antimicrobial peptides, namely PGLa [5], gramicidin S [6], temporin A [7], temporin L [7], as well as the cell penetrating peptides SAP [6,8], and transportan-10 [9] in their functionally relevant membrane-bound states. Moreover, CF_3-Bpg has also been used to investigate intermolecular interactions of the coiled-coil peptide pair VPE/VPK [10]. In fact, during the last 3 years, CF_3-Bpg proved to be the optimal label for solid-state ^{19}F NMR structure analysis of membrane-bound peptides [11]. The main advantage of CF_3-Bpg is the stereochemical stability compared to CF_3-Phg, which racemizes extensively under basic conditions employed during solid phase Fmoc peptide synthesis, and which has led to difficulties in numerous cases where it was impossible to separate the resulting peptide epimers by HPLC [12]. This problem is absent when using CF_3-Bpg, as it does not racemize upon incorporation into peptides. Along with increasing the final product yield and eliminating laborious epimer separation and assignment, this makes CF_3-Bpg an ideal ^{19}F NMR label. The growing interest in CF_3-Bpg prompted us to find a practical procedure for its large-scale production. Here, we report an optimized synthesis of CF_3-Bpg, which allows its preparation in multigram quantities.

2. Results and discussion

In our original work, the laboratory-scale synthesis of CF_3-Bpg [1] starting from **2** (Scheme 1) resulted in 1 g of the final product in 35% overall yield, which shall serve as a reference point for the up-scaling process.

With the intention to lower the costs of the synthesis where possible, each step was carefully checked. Thus, the synthetic step "b" (Scheme 1) had formerly been performed according to the original procedure reported by Adcock and Gack [13], i.e. 2.2 equiv.

* Corresponding author at: Department of Chemistry, Kyiv National Taras Shevchenko University, Vul. Volodymyrska 64, 01033 Kyiv, Ukraine.
Tel.: +380 44 2393315; fax: +380 44 2351273.
E-mail address: pashamk@gmx.de (P.K. Mykhailiuk).

0022-1139/$ – see front matter. Crown Copyright © 2009 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jfluchem.2009.10.004

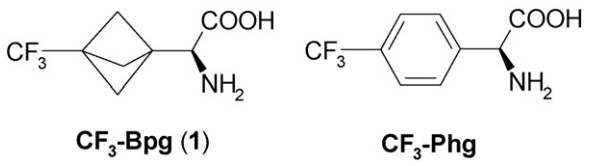
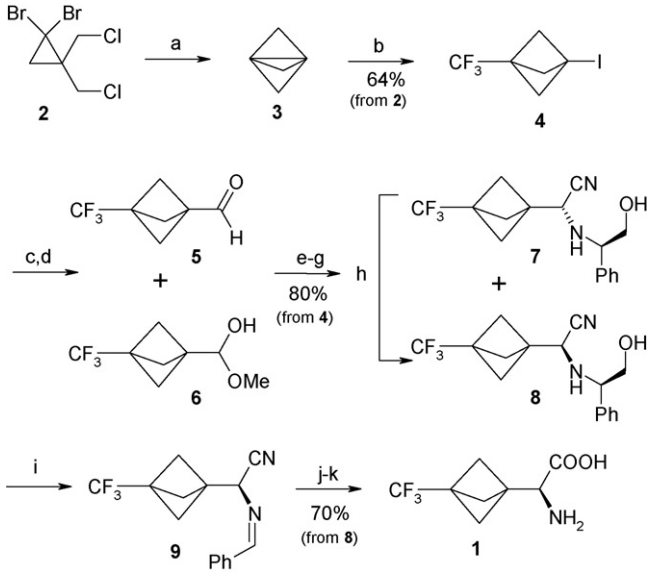


Fig. 1. Structures of the ^{19}F NMR labels CF_3-Bpg (**1**) and CF_3-Phg.



Scheme 1. Synthetic scheme used in the initial synthesis of CF_3-Bpg [1]. Reagents and conditions: (a) 2.4 equiv. MeLi, pentane, −78 °C, 0.5 h; (b) 2.2 equiv. CF_3I, pentane, rt, 20 h; (c) 1.1 equiv. *t*-BuLi, Et_2O, −78 °C, 1 h; (d) 4 equiv. HCO_2Me, Et_2O, −78 °C, rt, 3 h; (e) 1 equiv. (*R*)-α-phenylglycinol, CH_2Cl_2, rt, 2 h; (f) 3 equiv. Me_3SiCN, rt, 10 h; (g) chromatographic separation; (h) MeOH, reflux, 3 h; (i) 1.4 equiv. $Pb(OAc)_4$, CH_2Cl_2, 0 °C, 5 min; (j) 20% HCl, reflux, 2 h; (k) chromatography on Dowex-50.

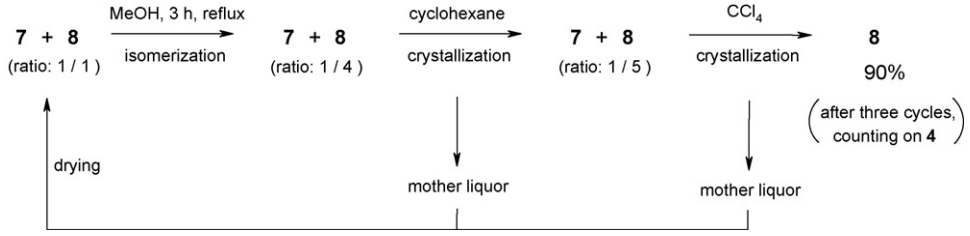
Table 1
Isolated yields of **4** at different CF_3I/propellane ratios, relative to **2**. The value marked grey was used in the large-scale synthesis.

CF_3I/propellane (mol. ratio)	2.2	1.7	1.5	1.1	0.8
Isolated yield of **4** (%)	64	62	63	62	54

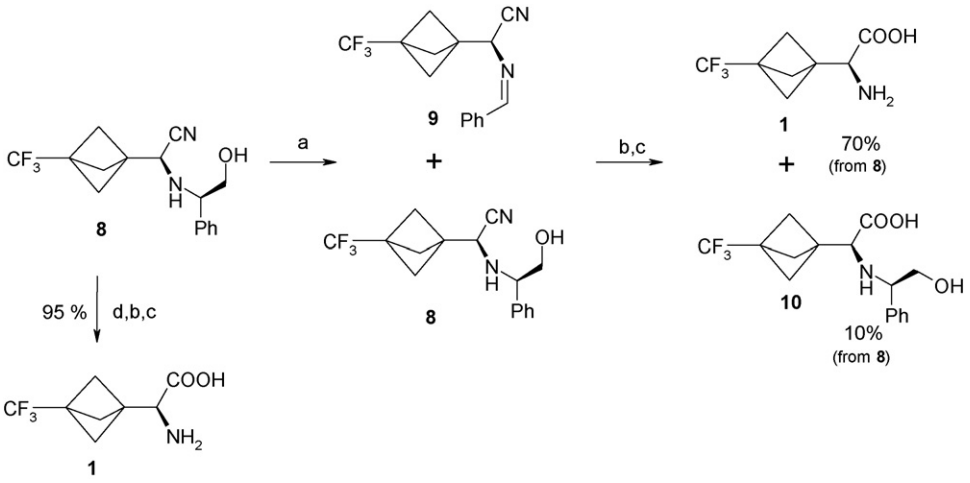
of CF_3I were added to the solution of 1 equiv. of propellane in Et_2O. Since CF_3I is a rather expensive chemical (448.5€ for 100 g of CF_3I in Sigma–Aldrich, 2009), a series of experiments were performed using different CF_3I/propellane ratios in order to diminish the amount of CF_3I (Table 1). Indeed, using only half as much CF_3I (1.1 equiv. instead of 2.2 equiv.) significantly lowered the cost without significantly compromising the yield (62% instead of 64%).

The next challenge encountered on the large-scale synthesis of aminonitrile **8**. A 1:1 mixture of **7** and **8** is obtained in the Strecker reaction of **5** and **6** with (*R*)-phenylglycinol (steps "e-g", Scheme 1) [1]. The individual components of the mixture can be separated by flash chromatography. However, the very small difference in the R_f values (∼0.05) made purification of **8** rather laborious and inefficient: to completely separate 5 g of the mixture **7** and **8**, more than 1.5 kg of Silica gel Merck 60 was needed. Obviously, to separate large quantity of **7** and **8** (>100 g), a much more efficient procedure had to be applied. Therefore, extensive experimentation was performed to find conditions to isolate **8** by means of crystallization.

First, the 1/1 mixture of **7** and **8** was isomerized in refluxing MeOH, to give an isomer ratio (**7**/**8**) of about 1/4, as previously observed [1]. Next, this mixture was crystallized from cyclohexane, which increased the ratio to ∼1/5 (Scheme 2). Remarkably, further crystallizations from cyclohexane did not improve this ratio. However, a second crystallization from CCl_4 provided **8** already as a single diastereomer (Scheme 2). The mother liquors from the two crystallization batches were combined, dried, and subjected to isomerization in MeOH to re-establish the initial compound ratio (**7**/**8** = ∼1/4). Threefold repetition of the isomerization–crystal-



Scheme 2. Optimized double crystallization procedure for the isolation of **8** from the mixture **7**/**8**.



Scheme 3. Reagents and conditions: (a) original procedure: 1.4 equiv. $Pb(OAc)_4$, CH_2Cl_2, 0 °C, 5 min; (b) 20% HCl, reflux, 2 h; (c) chromatography on "KU-2"; (d) optimized procedure: 1.5 equiv. $Pb(OAc)_4$, MeOH/CH_2Cl_2 (1/1), 0 °C, 15 min.

lization cycle provided **8** in 90% overall yield (from **4**), being even higher than that obtained initially by the original isomerization-chromatography approach (80%, Scheme 1). Notably, direct crystallization of **7**/**8** (1/4) from CCl_4 did not afford pure **8**.

The last step of the synthesis was the conversion of **8** into the target amino acid **1** via oxidation with $Pb(OAc)_4$. At the onset we failed to obtain **1** in a yield better than 70% [1]. Later, we reasoned, that it may happen because of the incomplete oxidation of **8** into **9** (Scheme 3). This assumption was subsequently proven by isolating the amino acid **10** (10% yield from **8**) along with **1** during purification of the reaction mixture. Obviously, **10** had formed as a result of acidic hydrolysis of the C≡N group of the residual non-oxidized **8** (Scheme 3). Indeed, the yield of **1** was significantly improved to 95%, simply by using 1.5 equiv. of $Pb(OAc)_4$ and increasing the reaction time to 15 min.

3. Conclusions

We have optimized the initially reported synthetic procedure for CF_3-Bpg. Conditions for isolating the key intermediate **8** from the mixture **7**/**8** by double crystallization were found. Using the optimized protocol, 100 g of CF_3-Bpg were obtained. The overall yield of CF_3-Bpg was increased from 35% to 53%.

4. Experimental

4.1. General

Solvents were purified according to standard procedures. Compound **2** was prepared according to the literature procedure [14]. All other materials were purchased from Fluka and Enamine. Melting points are uncorrected. ^{1}H-, ^{13}C- and ^{19}F NMR spectra were recorded on a Varian Unity Plus 400 spectrometer (at 400.4, 100.7 and 376.7 MHz, respectively). Chemical shifts are reported in ppm downfield from TMS (^{1}H, ^{13}C) or $CFCl_3$ (^{19}F) as internal standards. IR spectra were obtained on a Hewlett Packard UR 20 spectrometer. Mass spectra were recorded on an Agilent 1100 LCMSD SL instrument by chemical ionization (CI).

4.2. Preparation of 1-iodo-3-(trifluoromethyl)bicyclo[1.1.1]pentane (4)

2 (160 g, 0.54 mol) and absolute pentane (200 mL) were placed in a 2-L Favorsky apparatus in argon atmosphere. The suspension was cooled to −78 °C and 1.6 M solution of MeLi in Et_2O (800 mL, 1.28 mol) was added dropwise during 20 min under stirring. The mixture was allowed to warm to 0 °C and stirred for 1 h at this temperature. Thereafter, the mixture of Et_2O, pentane and propellane was distilled under reduced pressure into another 2-L vessel (thick glass) cooled by liquid nitrogen. According to Mondanaro and Dailey [15], the yield of propellane ranges from 75% to 88% (up to 0.48 mol). 2-L receiver, still cooled by liquid nitrogen, was disconnected from the Favorsky apparatus, and filled with CF_3I (105 g, 0.54 mol). The vessel was closed with a septum, in which a syringe with a rubber balloon was injected. Then, the flask was allowed to warm up to −20 °C, whereby the balloon was inflated. The syringe was disconnected, and the septum was additionally fixed on the flask by a metal clamp. The flask was left in the dark for 3 days at rt. Next, the solvent was slowly removed in vacuum on the rotary evaporator without heating (the product is very volatile). Upon evaporating, the temperature inside the flask decreased to −10 °C. It normally took 2–3 h to remove the solvent. **4** was obtained as white solid (87 g, 0.33 mol, 62% yield calculated on **2**). It had to be consumed right away, due to decomposition upon storage (after 2 weeks in the dark at 0 °C, **4** is completely decomposed). We normally used **4** the next day after isolation.

4.3. Preparation of 2(S)-{[(1R)-2-hydroxy-1-phenylethyl]amino}-2-[3-(trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetonitrile (8)

The crude mixture **7**/**8** (∼1/4) was prepared from **4** as previously described. Next, it was filtered through silica gel to remove minor viscous impurities. The obtained isomers **7**/**8** (100 g) were dissolved in cyclohexane (400 mL) upon heating, and then left to stand at rt for 2 h. The white solid was filtered to provide 92 g of **7**/**8** (∼1/5). The obtained amount of **7**/**8** was dissolved in CCl_4 (550 mL) upon heating, and left at room temperature for ∼30 min. To the warm solution (at this moment crystallization has not yet started), several crystals of pure **8** were added, and the mixture was left at rt overnight. White solid was filtered to afford pure **8** (52 g). Mother liquors from two crystallizations were combined and evaporated. The residue was dissolved in MeOH (500 mL) and refluxed for 3 h. The solvent was evaporated and the residue (**7**/**8**, ∼1/4) was submitted again to double crystallization. Repetition of the isomerization–crystallization procedure for three times provided 90 g of pure **8**.

4.4. Preparation of (2S)-2-amino-2-[3-(trifluoromethyl)bicyclo[1.1.1]pent-1-yl]ethanoic acid (1)

$Pb(OAc)_4$ (214 g, 0.48 mol) was added to a solution of **8** (100 g, 0.32 mol) in CH_2Cl_2/MeOH (3 L, 1/1). After being stirred at 0 °C for 15 min, the reaction was poured into a saturated aq. solution of $NaHCO_3$ (4 L). The resulting insoluble material was removed by filtration and washed with CH_2Cl_2 (2 L). Organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 1 L). The combined organic phases were evaporated in vacuum to give Schiff base **9** as yellow oil. It was dissolved in aq. HCl (6 M, 6 L), and refluxed for 6 h. After cooling, the reaction mixture was washed with Et_2O (3 × 1 L) and the aqueous layer was evaporated to produce the white solid. Afterwards, the residue was dissolved in H_2O (∼500 mL), neutralized with aq. NaOH (0.3 M) and submitted to cation exchange resin chromatography on "KU-2". The column was washed with water. Then, elution with aq. NH_3 (10%) was performed. Evaporation of the eluate afforded **1** (64 g, 0.30 mol, 95%) as a white solid.

If the reaction is carried out for 5 min instead of 15 min, the yield of **1** decreases to 70%, and another product **10** (10%) could be isolated as well. It was obtained as a second fraction in the ion exchange chromatography, following **1**.

4.5. (2S)-{[(1R)-2-hydroxy-1-phenylethyl]amino}[3-(trifluoromethyl)bicyclo[1.1.1]pent-1-yl]acetic acid (10)

m.p. 217–219 °C. $[\alpha]_D^{20} = -4.8$ (c = 11 mg/mL, CH_3OH).

^{1}H NMR (400 MHz, D_2O), δ: 7.51 (m, 5H, Ph), 4.32 (dd, J = 7.6, 5.0 Hz, 1H, C*H*CH_2), 3.84 (dd, J = 12.0, 5.0 Hz, 1H, CHC*H*H), 3.78 (dd, J = 12.0, 7.6 Hz, 1H, CHCH*H*), 3.73 (s, 1H, *CH*COOH), 1.95 (s, 6H, (CH_2)$_3$).

^{19}F NMR (377 MHz, D_2O), δ: −75.50 (s, CF_3).

^{13}C NMR (100 MHz, D_2O), δ: 170.52 (s, *C*OOH), 132.79 (s, *tert-C*, Ph), 128.07 (s, *C*H, Ph), 127.96 (s, *C*H, Ph), 125.79 (s, *C*H, Ph), 121.34 (q, $^1J_{C-F}$ = 271.0 Hz, CF_3), 61.44 (s, CH_2Ph), 55.05 (s, *C*HCOOH), 53.41 (s, CH_2OH), 46.39 (q, $^3J_{C-F}$ = 2.0 Hz, (CH_2)$_3$), 36.09 (q, $^4J_{C-F}$ = 2.0 Hz, CH_2*C*CH), 34.59 (q, $^2J_{C-F}$ = 39.0 Hz, *C*CF_3).

MS (m/z): 330 (M+1).

Acknowledgements

PM is very grateful to Dr. Magda Tsapko for NMR measurements, and all authors thank the Alexander von Humboldt Foundation for supporting their institute partnership.

References

[1] P.K. Mykhailiuk, S. Afonin, A.N. Chernega, E.B. Rusanov, M.O. Platonov, G.G. Dubinina, M. Berditsch, A.S. Ulrich, I.V. Komarov, Angew. Chem. 118 (2006) 5787–5789; Angew. Chem. Int. Ed. 45 (2006) 5659–5661.
[2] R.W. Glaser, C. Sachse, U.H.N. Dürr, P. Wadhwani, A.S. Ulrich, J. Magn. Reson. 168 (2004) 153–163.
[3] R.S.W. Gläzer, C. Sachse, U.H.N. Dürr, P. Wadhwani, S. Afonin, E. Strandberg, A.S. Ulrich, Biophys. J. 88 (2005) 3392–3397.
[4] S.L. Grage, A.V. Suleymanova, S. Afonin, P. Wadhwani, A.S. Ulrich, J. Magn. Reson. 203 (2006) 77–86.
[5] S. Afonin, P.K. Mykhailiuk, I.V. Komarov, A.S. Ulrich, J. Pept. Sci. 13 (2007) 614–623.
[6] P.K. Mykhailiuk, PhD Thesis, University of Karlsruhe, 2008.
[7] D. Tiltak, PhD Thesis, University of Karlsruhe, 2009.
[8] P.K. Mykhailiuk, S. Afonin, G.V. Palamarchuk, O.V. Shishkin, A.S. Ulrich, I.V. Komarov, Angew. Chem. 120 (2008) 5849–5851; Angew. Chem. Int. Ed. 45 (2008) 5765–5767.
[9] B. Ehni, Diploma Thesis, University of Karlsruhe, 2009.
[10] M. Salwiczek, B. Koksch, manuscript in preparation.
[11] P. Wadhwani, E. Strandberg, in: I. Ojima (Ed.), Fluorine in Medicinal Chemistry and Chemical Biology, Wiley-Blackwell, 2009, pp. 463–493.
[12] A. Eisele, Diploma Thesis, Karlsruhe University, 2007.
[13] J.L. Adcock, A.A. Gack, J. Org. Chem. 57 (1992) 6206–6210.
[14] K. Mondanaro Lynch, W.P. Dailey, J. Org. Chem. 60 (1995) 4666–4668.
[15] K.R. Mondanaro, W.P. Dailey, Org. Synth. 10 (2004) 658; Org. Synth. Coll. 75 (1998) 98.