EurJOC
European Journal of Organic Chemistry

A Journal of ChemPubSoc Europe

Accepted Article

Title: The formamide as an unconventional amine protecting group for PET radiochemistry

Authors: Jimmy E Jakobsson, Gaute Grønnevik, Waqas Rafique, Karoline Hartvig, and Patrick Johannes Riß

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201800554

Link to VoR: http://dx.doi.org/10.1002/ejoc.201800554

Supported by ACES Asian Chemical Editorial Society

WILEY-VCH

10.1002/ejoc.201800554

WILEY-VCH

The formamide as an unconventional amine protecting group for PET radiochemistry

Jimmy Erik Jakobsson,[a] Gaute Grønnevik,[ab] Waqas Rafique,[a] Karoline Hartvig[a] and Patrick Johannes Riss*[abc]

Abstract: We developed a versatile, rapid and robust high yielding radiochemistry adapted protocol utilising formamides as masking groups for secondary and tertiary amines. Selective reducing conditions were devised using borane reagents. In this protocol formamide functionalities were found to have an orthogonal reactivity to most other carbonyl functions, while effectively protecting amines from oxidative degradation. We exemplify the newly developed methodology by synthesising a μ-opioid PET radiotracer and a dopamine PET radiotracer analogue.

Introduction

Molecular imaging is a powerful non-invasive tool that can visualise biological pathways inside living organisms. An example of molecular imaging is positron emission tomography (PET[1]) which is used for both diagnosing various disease states e.g. cancer and Alzheimer's disease and for unravelling biological mechanisms *in vivo*. PET imaging is based on positron emitting isotopes. Most often, the positron emitting nuclide of choice is fluorine-18 due to its excellent decay characteristics and convenient half-life (109.7 min). Fluorine-18 is produced in a cyclotron via the $^{18}O(p,n)^{18}F$ nuclear reaction and obtained as $^{18}F^-$ in an aqueous solution. The fluoride is typically retained on an anion exchange cartridge, reformulated with a phase transfer catalyst and azeotropically dried to enhance the nucleophilicity. Production of radiotracers for clinical use require rigorous quality controls, therefore it is of uttermost importance to limit the use of potentially toxic reagents as far as possible to minimise the number of chemicals that needs to be tested for to ease clinical translation.

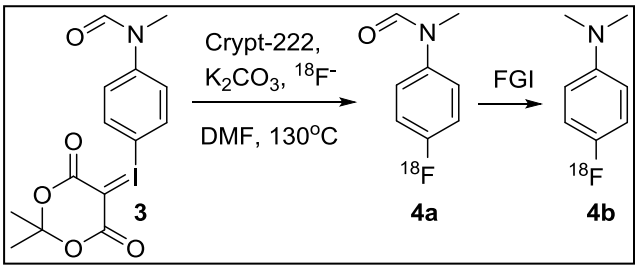
There is a strong demand for new radiotracers and consequently new means of incorporating fluorine-18 into both aromatic and aliphatic systems are needed. Significant effort has been made to develop new labelling methodologies,[2] however, little attention has been given to increase the functional group tolerance of said methods in order to allow for radiosynthesis of final products in one step. The most abundant fluorinated motif in drug compounds are aryl fluorides, while activated systems are readily radiofluorinated via a nitro-to-fluoro substitution reaction, deactivated substrates require other types of precursors often in combination with a transition metal catalyst selected from Cu, Ni or Pd.[2a, 2b] However, the conditions developed for the purpose proceed in significantly lower yield or not at all in presence of free amines[2b, 2f, 2g](ESI). This makes necessary the use of protective groups during labelling followed by deprotection. Primary and secondary amines can readily be masked as carbamates, however, tertiary amines cannot. In another example, synthesis of hypervalent iodanes as precursors, free amines are frequently oxidised and the available strategy for avoiding oxidation, protonation via strong acids only works occasionally and is incompatible with sensitive functional groups. Hypervalent iodane nucleofuges are the only transition metal free methodology that consistently provides radiofluorination of both deactivated and sterically hindered positions in good yields.[3]

We believe that developing a protective group approach adapted to radiochemistry will improve the predictability and usefulness of iodonium ylides as versatile radiofluorination precursors for accessing complex substrates, as well as benefit transition metal catalysed radiochemistry.

Results and Discussion

We surmised that masking amines as formamides would extinguish their nucleophilicity, allow synthesis of primary, secondary or tertiary amines and remove sensitivity to oxidation, thus providing a versatile strategy. Functional group interconversions such as hydrolysis or reduction further amplify the scope of products. Radiofluorination of all non-activated substrates was achieved from the corresponding iodonium ylide precursor in excellent conversion (87±10%, n=114, radioTLC) with high reproducibility in between both, individual runs and batches under standard radiofluorination conditions[3a] and without radioactive side products.

Scheme 1 Radiofluorination performed using ylide (4.0 mg, 10 μmol), crypt-222 (10 mg, 27 μmol) K_2CO_3 x 1.5 H_2O (1.84 mg, 11 μmol) and $^{18}F^-$ (400 MBq) in DMF (1 ml) at 130 °C for 20 min. Crypt-222 = 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane



A standard workup included dilution of the reaction mixture with water and purification using a Chromabond HR-P C_{18} cartridge to remove residual precursor and DMF left from the radiofluorination reaction (78±3% recovered radiotracer in ~95% RCP, n=3, radioTLC). Use of other solvents produces regioisomeric mixtures and reduced yield.

First we focussed on devising reduction conditions to convert formamide model compounds into tertiary amines. Prior to amide reduction, residual water has to be removed, cartridges packed with various drying agents and azeotropic distillation was evaluated as alternatives (Table 1).

[a] Realomics Strategic Research Initiative, Department of Chemistry, Faculty for Mathematics and Natural Sciences, University of Oslo, Norway. E-mail: Patrick.riss@kjemi.uio.no
[b] Norwegian Medical Cyclotron AS, Nydalen, Oslo, Norway
[c] Department of Surgery and Neuroscience, OUS-Rikshospitalet HF, Oslo, Norway

Table 1. Formamide **4a** to 9-BBN in THF (50 µmol) at 90 °C in THF (0.5 ml) for 10 min. RCY reported is based on radioHPLC.

Entry	Cartridge	Elution solvent	RCY(%)
1	None	THF	0
2	None	MeCN	68[a]
3	Na_2SO_4	THF	0[b]
4	$MgSO_4$	THF	0[b]
5	$MgSO_4$	DCM	0[b]
6	Silica	THF/DCM 9:1	92±2 (3)[c]
7	Silica	DCM	0[d]

[a] azeotropic distillation 33% of radioactivity lost during evaporation (85 deg, 1 x MeCN (1 ml) addition) [b] handmade plug [c] 10±6% lost during evaporation, n=45 (50 °C, 15 min) [d] radiotracer fail to elute through Si cartridge. RCY is based on radioHPLC analysis. 9-BBN = 9-Borabicyclo[3.3.1]nonane.

The drying efficiency was evaluated by comparing the subsequent reduction yield using 9-BBN that under non-radioactive stoichiometric anhydrous conditions in THF provided good reduction yields of no carrier added **4a**. Removal of trace water via azeotropic distillation with MeCN yielded a successful reduction (68%) (Table 1, entry 2), however, a large portion of the radioactivity was lost to evaporation and the methodology deemed unsuitable for our model substrate. We evaluated common drying agents and neither sodium sulfate nor magnesium sulfate provided sufficient dryness (Table 1, entry 3-5). A silica cartridge was evaluated and pleasantly excellent yields of the amine were achieved, THF was added to increase the elution strength of the solvent mixture (Table 1, Entry 6-7). Enthused by the excellent reduction yield, additional reducing agents were evaluated to see if further improvement could be achieved (Table 2).

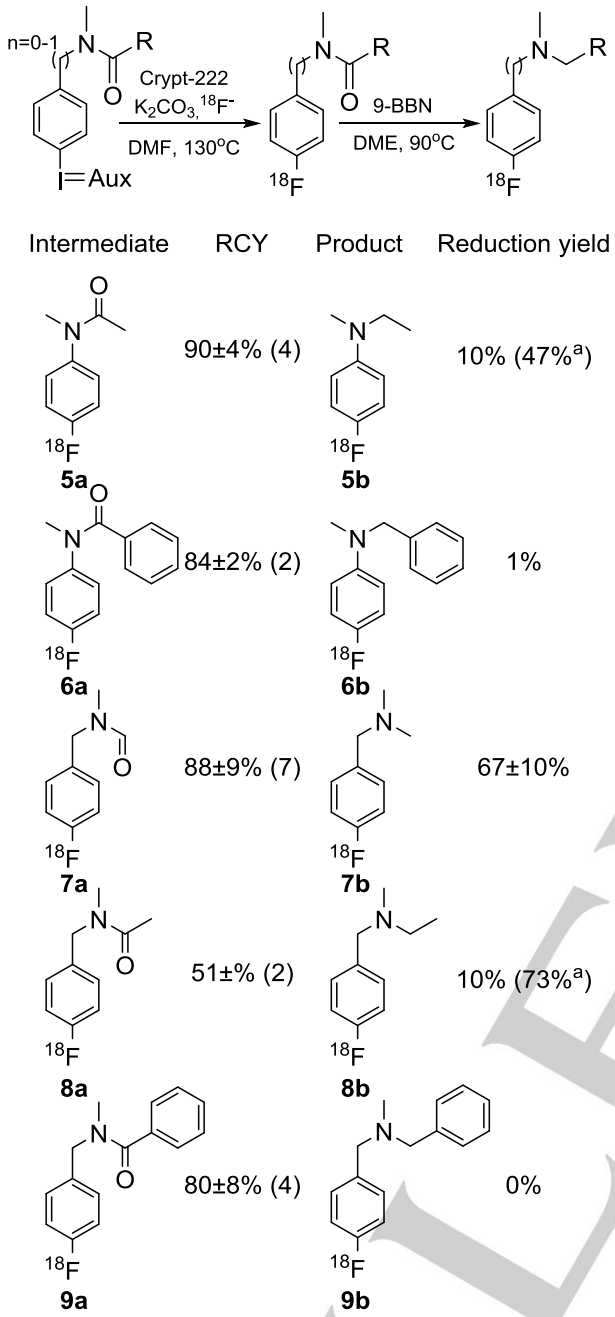
Table 2. Formamide **4a** to 0.5 M 9-BBN in THF (50 µmol) at 90 °C in solvent (0.5 ml) for 10 min. RCY is based on radioHPLC analysis.

Entry	Reagent	Solvent	RCY
1	Phenylsilane	THF	0 (1)
2	Poly(methylhydrosilane)	THF	0 (1)
3	BH_3•SMe_2	THF	90±1 (3)[a]
4	9-BBN	THF	92±2 (3)[b]
5	9-BBN	DCE	89±2 (3)
6	9-BBN	MeCN	67±3 (3)
7	9-BBN	iPr_2O	92±0 (3)
8	9-BBN	DME	87±4 (3)
9	9-BBN	DME	31 (1)[c]
10	9-BBN	DME	70±3 (2)[d]
11	9-BBN	DME	45±4 (2)[e]
12	9-BBN	DME	52±2 (3)[f]

[a] Reactions yielding no or very low conversions are excluded [b] Entry is identical to Table 1, Entry 6 [c] 1 minute reaction time [d] 75°C [e] 60°C [f] 30 µmol 9-BBN. RCY is based on radioHPLC analysis. 9-BBN = 9-Borabicyclo[3.3.1]nonane. For radioactivity balance see ESI.

Silanes produced no conversion (Table 2, entry 1-2). Borane dimethylsulfide complex afforded comparable conversion to 9-BBN but was more difficult to handle due to higher volatility. We also noted an undesired variability in the reaction outcome (Table 2, entry 3) with occasional experiments yielding no or very low conversion. Further investigating solvent effects it was found that ethers produced the highest reduction yields (Table 2, Entry 4-8). Interestingly all reactions utilising DME as solvent have radioHPLC and radioTLC chromatograms devoid of an unidentified radioactive side product present for all other solvents inconveniently appearing in between starting amide and product which could render final purification difficult. It was hypothesised that DME facilitate the reduction step via a bidentate coordination to boron, however, adding small quantities of DME or TMEDA to THF did not produce similar results (ESI). The reaction proceeded very quickly, after 1 minute 31% (Table 2, Entry 9)) of the substrate had been reduced, whereas near complete reduction (92±0%) took only ten minutes. Reducing the temperature to 75 °C and 60 °C reduced the yields to 70±3% and 45±4%, respectively, after ten minutes (Table 2, Entry 10-11). Reducing the reagent loading to 3 µmol reduced the yield of **4b** to 52±2%**.**

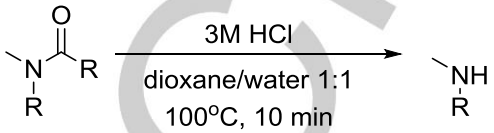
Curious how well the method might translate into aliphatic formamides and if selective reduction of formamides over acetamides and benzamides can be expected we investigated the scope of amide substrates. We synthesised and radiofluorinated the corresponding iodonium ylides of substrate **5a-9a** in good to excellent yields (Figure 1).



Scheme 1. Radiofluorination performed using ylide (10 µmol), crypt-222 (10 mg, 27 µmol) K_2CO_3 x 1.5 H_2O (1.84 mg, 11 µmol) and $^{18}F^-$ (100-500 MBq) in DMF (1 ml) at 130 °C for 20 min. Amide reduction using 0.5 M 9-BBN in THF (50 µmol) at 90 °C in DME (0.5 ml) for 10 min. RCY is based on radioTLC analysis and reduction yield is based on radioHPLC analysis. [a] 260 µmol 9-BBN, 120 °C, 20 min. 9-BBN = 9-Borabicyclo[3.3.1]nonane. Crypt-222 = 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane.

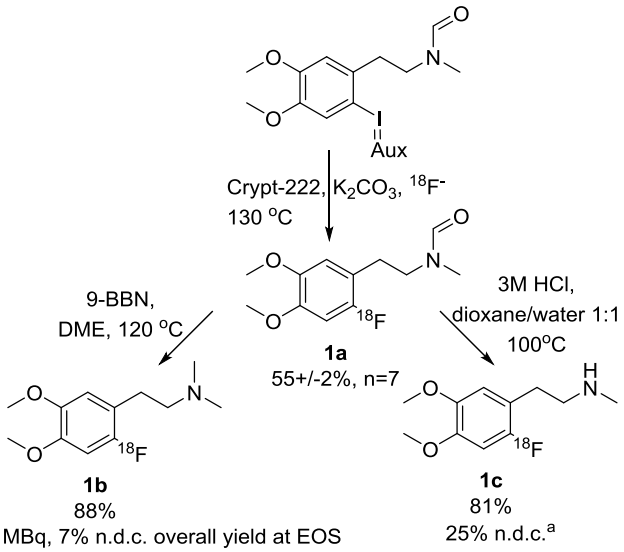
As expected formamide **7a** was readily reduced under standard conditions producing amine **7b** (67±10%) whereas acetamides **5a** and **8a** were reduced in 10% RCY to **5b** and **8b** respectively under standard reaction conditions, additional reagent loading, increased temperature and duration achieved 47% and 73% RCY of **5b** and **8b** respectively. The corresponding benzamides **6a** and **9a** proved almost inert to the reaction conditions producing only traces (1%) of the amines **6b** and **9b.** The results bode well for achieving selective reduction of formamides over acetamides and benzamides.

Table 3. 100 degrees in dioxane/water 1:1, 10 min. RCY reported is based on radioHPLC analysis.



Entry	Substrate	Conditions	RCY
1	4	3M HCl	78±1 (3)
2	7	3M HCl	61±5 (3)

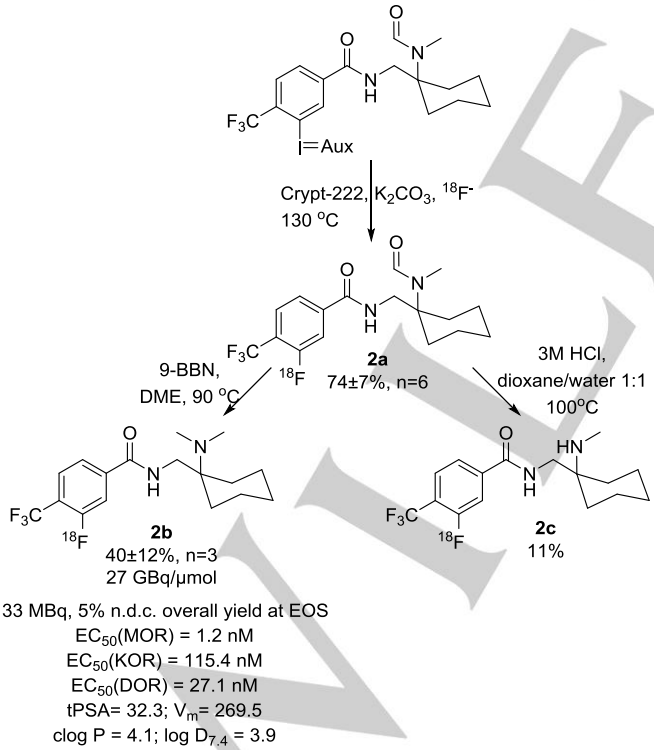
Inspired by the straightforward synthesis of both precursors and radiotracers (**4a-9a**) it was investigated further if formamides could serve as an alternative protecting group to carbamates the typical secondary amine-protecting group. Monomethylated amines are also a common motif in drug molecules,[4] more metabolically stable than dimethyl amines,[5] which sometimes lead to a desmethyl metabolite troublesome in PET imaging. A convenient access to mono- and dimethyl-analogues of basic amines could allow for both radiotracer candidates to be made from the same precursor. Pleasantly a high yield of the monomethylated derivatives was achieved within 10 minutes by hydrolysis of the formamide in 3M HCl (Table 3, Entry 1 and 4), extended time or harsher conditions did not improve the reduction (Table 3, entry 2-3).



Scheme 2. Radiofluorination of phenethylamide **1a** and subsequent formamide reduction or hydrolysis yielding potential dopamine analogue radiotracers **1b** and **1c**. RCY for **1a** is based on radioTLC analysis. RCY for **1b-c** is based on radioHPLC analysis. For radioactivity balance see ESI. [a] yield is calculated from a crude sample using analytical HPLC and RCP.

Satisfied with the method development we selected two compounds for proof of principle, phenethylamine **1b** and μ-opioid agonist **2b** (Scheme 2), as target amines to demonstrate the usefulness of the developed methodology. The difficult to access electron rich and sterically hindered amide **1a** was radiofluorinated in good yield (55±2%, n=7)**.** Reduction under standard conditions proceeded in a meagre 25% RCY, a minor reaction optimization by increasing the temperature to 120 °C and using twice the 9-BBN loading yielded the dimethyl radiotracer **1b** in 88% RCY in 10 minutes (7% overall n.d.c. yield was achieved after purification yielding 1b in >99% RCP after 76 minutes synthesis with two major UV active sideproducts). Hydrolysis of **1a** at 100 °C in 3M HCl yielded only 23% of **1c**. Increasing the temperature to 110 °C for 20 minutes yielded the desmethyl radiotracer **1c** in 81% with no side products (25% n.d.c. yield at EOS (60 min synthesis)).

To further demonstrate the power, versatility and limitations of the developed protocol we synthesised μ-opioid receptor radiotracer (**2b**) (Scheme 3), to test the selectivity of the reduction method. The non-activated and sterically hindered formamide **2a** was radiofluorinated in 74±7**%,** n=6**.** Reduction under standard conditions furnished dimethyl amine radiotracer **2b** in 40±12**%** (5% overall RCY was achieved in 96% RCP (radioHPLC) after silica cartridge purification (76 min synthesis). Increasing temperature or reagent loading yielded more complex reaction mixtures, possibly due to competing reduction of benzamide. Hydrolysis of amide (**2a**) produced radiotracer **2c** in (11%) but major degradation was observed, possibly due to hydrolysis of the benzamide. Lower temperature or lower concentration of HCl did not improve the yield.



Scheme 3. Radiofluorination of a formamide protected iodane and subsequent reduction or hydrolysis yielding selective μ-opioid receptor agonist radiotracers **2b** and **2c**. RCY for **2a** is based on radioTLC. RCY for **2b-c** is based on radioHPLC. For radioactivity balance see ESI.

Conclusions

We demonstrate a transition-metal free radiochemistry adapted protocol for masking secondary and tertiary amines to expand the available radiotracer substrate scope. We conclude that minor optimisations can be expected in between substrates and that the formamides can be either reduced or hydrolysed providing a convenient access to both dimethylamines and mono-methylated amines. Challenging to access radiotracer **1b-c** and **2b-c** were synthesised in sufficient overall yield for potential evaluation in imaging studies.

Acknowledgements

This work was partly supported by a personal grant to PJR (NFR ES 231553) and JJ thanks the Realomics SRI for a doctoral fellowship.

Keywords: Radiofluorination • hypervalent iodine • iodonium ylides • μ-opioid • protective groups

[1] P. W. Miller, N. J. Long, R. Vilar, A. D. Gee, *Angew Chem Int Ed Engl* **2008**, *47*, 8998-9033.

[2] a) N. Ichiishi, A. F. Brooks, J. J. Topczewski, M. E. Rodnick, M. S. Sanford, P. J. Scott, *Org Lett* **2014**, *16*, 3224-3227; b) A. V. Mossine, A. F. Brooks, K. J. Makaravage, J. M. Miller, N. Ichiishi, M. S. Sanford, P. J. Scott, *Org Lett* **2015**, *17*, 5780-5783; c) T. L. Ross, J. Ermert, C. Hocke, H. H. Coenen, *J Am Chem Soc* **2007**, *129*, 8018-8025; d) L. Mu, C. R. Fischer, J. P. Holland, J. Becaud, P. A. Schubiger, R. Schibli, S. M. Ametamey, K. Graham, T. Stellfeld, L. M. Dinkelborg, L. Lehmann, *Eur J Org Chem* **2012**, 889-892; e) P. J. Riss, V. Ferrari, L. Brichard, P. Burke, R. Smith, F. I. Aigbirhio, *Org Biomol Chem* **2012**, *10*, 6980-6986; f) E. Lee, J. M. Hooker, T. Ritter, *J Am Chem Soc* **2012**, *134*, 17456-17458; g) A. S. Kamlet, C. N. Neumann, E. Lee, S. M. Carlin, C. K. Moseley, N. Stephenson, J.

M. Hooker, T. Ritter, *PLoS One* **2013**, *8*, e59187.

[3] a) J. E. Jakobsson, G. Gronnevik, P. J. Riss, *Chem Commun (Camb)* **2017**, *53*, 12906-12909; b) N. Satyamurthy, J. R. Barrio, WLO2010117435(A2), **2010**.

[4] W. E. Klunk, H. Engler, A. Nordberg, Y. Wang, G. Blomqvist, D. P. Holt, M. Bergstrom, I. Savitcheva, G. F. Huang, S. Estrada, B. Ausen, M. L. Debnath, J. Barletta, J. C. Price, J. Sandell, B. J. Lopresti, A. Wall, P. Koivisto, G. Antoni, C. A. Mathis, B. Langstrom, *Ann Neurol* **2004**, *55*, 306-319.

[5] V. W. Pike, *Trends Pharmacol Sci* **2009**, *30*, 431-440.

10.1002/ejoc.201800554

WILEY-VCH

Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Text for Table of Contents

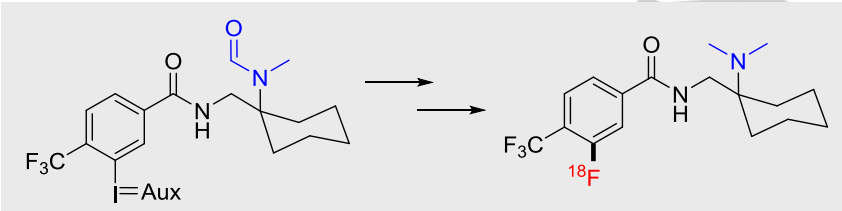
Key Topic*

((Insert TOC Graphic here: max. width: 5.5 cm; max. height: 5.0 cm; NOTE: the final letter height should not be less than 2 mm.))

*one or two words that highlight the emphasis of the paper or the field of the study

Layout 2:

COMMUNICATION



Formamide protection eases the application of recent aromatic fluorination methods.

Fluorination

*Jimmy E Jakobsson, Gaute Grønnevik, Patrick J Riss**

Page No. – Page No.

The formamide as an unconventional amine protecting group for PET radiochemistry

*one or two words that highlight the emphasis of the paper or the field of the study