Tetrahedron Letters xxx (xxxx) xxx

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthetic routes to treprostinil *N*-acyl methylsulfonamide

Christina Picken [a,b], Peter Laing [a,b], Lei Shen [a], Lucie H. Clapp [a,*], Steve Brocchini [b,*]

[a] Institute of Cardiovascular Sciences, University College London, Gower Street, London WC1E 6BT, UK
[b] School of Pharmacy, University College London, 29-39 Brunswick Square, London WC1N 1AX, UK

ARTICLE INFO

Article history:
Received 26 September 2019
Revised 15 November 2019
Accepted 19 November 2019
Available online xxxx

Chemical compounds:
Treprostinil (PubChem CID6918140)
Triol (PubChem CID11174966)

Keywords:
Prostacyclin
Treprostinil
Acylsulfonamide
Prodrug

ABSTRACT

The synthesis of the prodrug candidate, treprostinil *N*-acyl methylsulfonamide **5** was accomplished from treprostinil **2** utilising protecting group strategies. A more direct synthesis for the prodrug was also achieved using a treprostinil triol precursor **12** and bromoacetyl acylmethylsulfonamide **14**. The overall yield of treprostinil *N*-acyl sulfonamide **5** directly from the triol precursor **12** is similar to the protecting group strategies because deprotonation of the acidic proton in the bromoacetyl acylmethylsulfonamide **14** reduces electrophilicity. However, the more direct route using the treprostinil triol precursor holds greater promise as a strategy to prepare a wide range of treprostinil prodrug candidates. Treprostinil *N*-acyl methylsulfonamide prodrug **5** exhibited a 30-fold decrease in the potency at the human prostacyclin (IP) receptor compared to treprostinil **2** in an *in vitro* cyclic AMP assay.

Introduction

Pulmonary arterial hypertension (PAH) is a chronic inflammatory disease of the small pulmonary capillaries, characterised by vasoconstriction, cell proliferation and fibrosis [1] which increases pulmonary arterial pressure resulting in heart failure and ultimately death [2]. Reduced endogenous prostacyclin **1** is implicated in the aetiology and progression of PAH [3,4] leading to the use of prostacyclin **1** to treat PAH (see Fig. 1) [5–7]. Prostacyclin **1** acts at the prostacyclin (IP) receptor, also termed the prostaglandin I_2 receptor. Despite its potent vasodilatory, antiproliferative and anti-platelet properties, prostacyclin **1** is chemically and metabolically unstable [8–10].

Treprostinil **2** is a stable prostacyclin mimetic shown to alleviate symptoms and slow disease progression in patients (see Fig. 1) [11,12]. As with all prostacyclin analogues, treprostinil **2** has dose-limiting toxicities [13]. Treprostinil **2** can be administered by continuous ambulatory pump to provide a controlled dose through a subcutaneous catheter [14].

Despite improved clinical efficacy, the subcutaneous administration of treprostinil is associated with pain at the infusion site [15] which can lead to patients withdrawing from therapy [13].

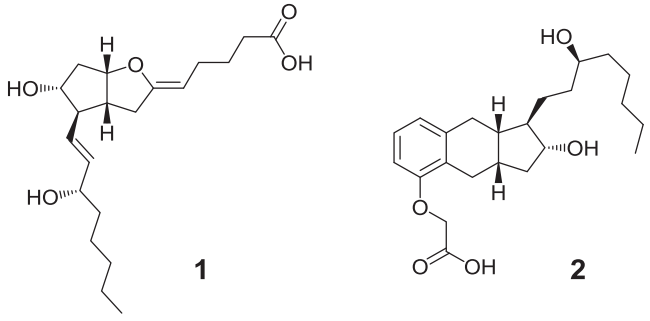


Fig. 1. Structures of endogenous prostacyclin **1** and treprostinil **2**.

Infusion site pain is principally attributed to the affinity of treprostinil at the IP receptor but may possibly involve additional prostanoid receptors such as the EP_2 and DP_1 receptors which are also located in the skin [16,17], and would be activated at similar concentrations to the IP receptor by treprostinil [9].

The presence of the treprostinil carboxylic acid moiety is important for maintaining biological activity at the IP receptor [18]. Several treprostinil carboxylic acid esters have been evaluated but no treprostinil prodrugs have been clinically registered [19–23], presumably due to the action of endogenous esterases leading to the premature release of treprostinil [24].

* Corresponding authors.
E-mail addresses: l.clapp@ucl.ac.uk (L.H. Clapp), s.brocchini@ucl.ac.uk (S. Brocchini).

https://doi.org/10.1016/j.tetlet.2019.151428
0040-4039/

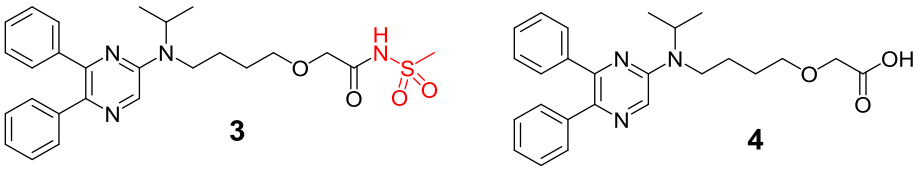


Fig. 2. Structure of the prodrug selexipag **3** that is cleaved by hepatic enzymes to give the metabolite ACT-333679 **4** which is a potent IP receptor agonist.

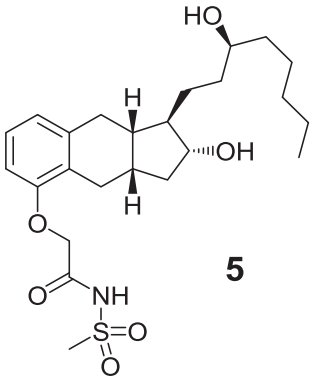


Fig. 3. Structure of treprostinil *N*-acyl methylsulfonamide **5**.

The orally administered non-prostanoid, *N*-acyl methylsulfonamide pro-drug called selexipag **3** (marketed as UPTRAVI) [25] had a 13-fold lower affinity for the IP-receptor than the active form of the drug (ACT-333679 **4**; Fig. 2) [26,27]. Upon absorption of selexipag **3** into the bloodstream, the *N*-acyl methylsulfonamide moiety is cleaved by hepatic carboxyesterases to unmask the active drug **4** exhibiting efficacy for treating PAH [28–30]. Selexipag **3** was designed to reduce side effects caused by the direct activation of IP receptors before absorption into the bloodstream [28].

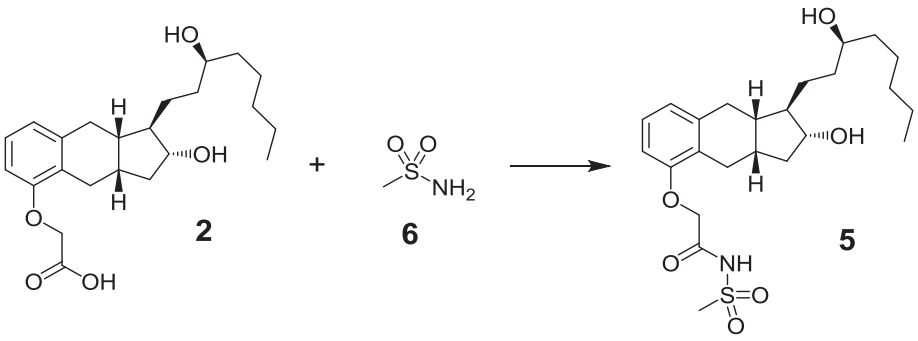
N-Acylsulfonamides are known to exhibit chemical and biological stability making them desirable candidates for prodrug structures [31]. We considered that a *N*-acyl methylsulfonamide form of treprostinil would combine the extended-release characteristics of a drug such as selexipag with the desirable pharmacological profile of treprostinil to reduce unwanted activity upon administration and ultimately achieve greater tolerability. We sought to prepare treprostinil *N*-acyl methylsulfonamide **5**, which we hypothesise will have lower activity at the IP receptor compared to treprostinil **2** (see Fig. 3).

Results and discussion

The preparation of treprostinil *N*-acyl methylsulfonamide **5** by directly coupling treprostinil **2** (50 mg scale) and methylsulfonamide **6** (Scheme 1) using either carbodimidazole (CDI) or *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) resulted in multiple products as determined by TLC analysis, which were difficult to isolate. ^{1}H NMR and HPLC analysis of the crude product mixtures suggested that a significant amount of treprostinil **2** had not undergone reaction. Efforts to prepare the *N*-hydroxysuccinimide ester of treprostinil were similarly unsuccessful (determined by TLC and ^{1}H NMR). A protecting group strategy appeared to be necessary to avoid possible side reactions due to the treprostinil secondary hydroxyls.

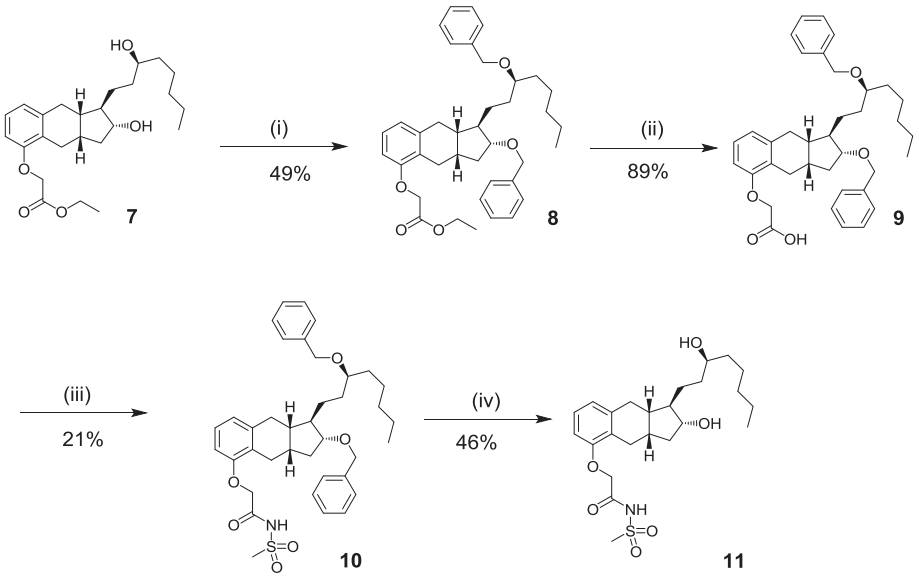
Treprostinil protecting group strategies have been described in the patent literature [32–35]. These strategies rely on the simultaneous protection of the carboxylic acid and the two secondary hydroxyls, which would then be followed by subsequent selective deprotection of the carboxylic acid. Several methods were evaluated (ESI Table S1) but did not generate sufficient yields of pure product. One strategy to per-trimethylsilylate treprostinil [35] using *N*,*O*-bis(trimethysilyl)acetimidate to give the tri-silylated treprostinil adduct, relied on the lability of the trimethylsilyl ester to allow coupling of methanesulfonamide **6** to give the desired treprostinil *N*-acyl methylsulfonamide **5**. A treprostinil-derived product was isolated but analysis by ^{1}H NMR did not confirm the presence of the desired product.

Attempts to directly benzylate the two hydroxyls in treprostinil **2** using an excess of different bases and benzyl bromide, resulted in no reaction or the formation of several products within which the desired bis-hydroxyl benzylate treprostinil product was observed in small amounts. To avoid competitive reactions with the treprostinil carboxylic acid moiety, treprostinil ethyl ester **7** could be prepared in good yield (88%) by Fisher esterification, allowing for benzylation of the secondary alcohols to again be examined (ESI Table S1). Benzylation of treprostinil and the corresponding ethyl ester proved difficult owing to the poor nucleophilicity of the secondary alcohols. It was found that when treprostinil ethyl ester **7** was treated with the Dudley reagent (2-benzyloxy-1-methylpridinium triflate) [36] dissolved in dichloromethane (DCM) the desired bis-benzyl treprostinil ethyl ester **8** (Scheme 2) was formed as a yellow oil in 49% yield following isolation by column chromatography. Following successful benzylation, direct benzylation of treprostinil using the Dudley reagent was also attempted and showed efficient conversion as determined by TLC.

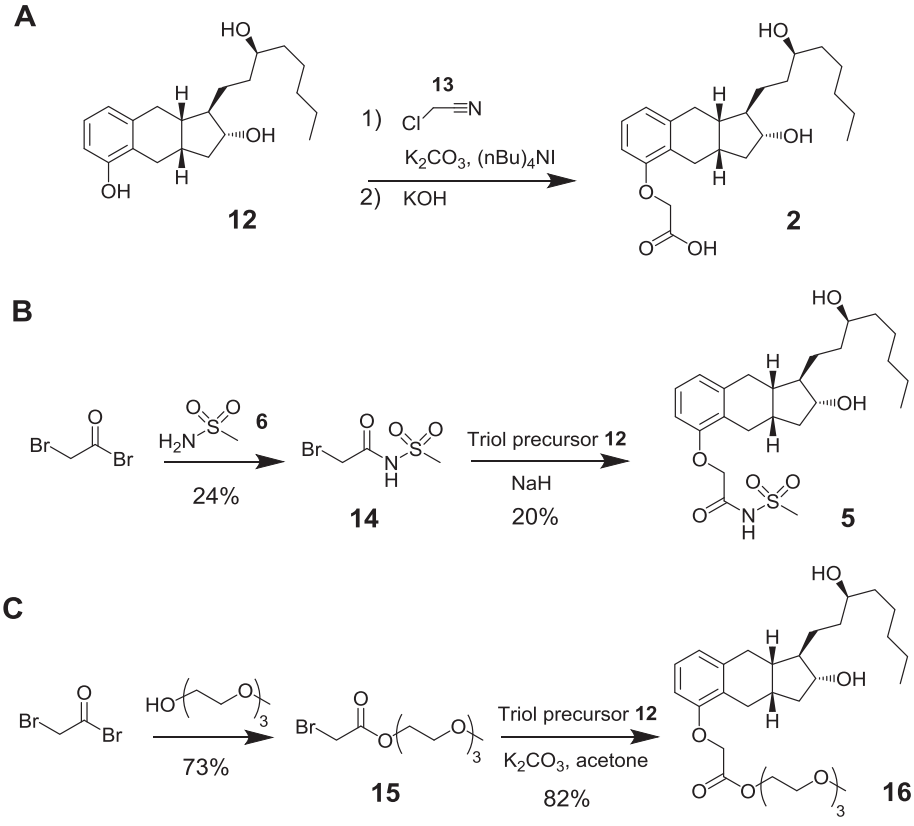


Scheme 1. Proposed direct conversion of treprostinil **2** to treprostinil *N*-acyl methylsulfonamide **5**.

Please cite this article as: C. Picken, P. Laing, L. Shen et al., Synthetic routes to treprostinil *N*-acyl methylsulfonamide, Tetrahedron Letters, https://doi.org/10.1016/j.tetlet.2019.151428



Scheme 2. Synthesis of treprostinil *N*-acyl methylsulfonamide **5** from treprostinil **2**. (i) Dudley reagent, MgO, trifluorotoluene; (ii) LiOH, MeOH/THF/water; (iii) methane sulfonamide **6**, carbodiimidazole, 1,8-Diazabicyclo[5.4.0]undec-7-ene, DCM; (iv) Pd/C, H_2, EtOH. Isolated yields are shown.



Scheme 3. Triol **12** has potential to add to the carboxylic acid head group and prodrug moiety in one step. (A) The synthesis of treprostinil **2** from the triol precursor **12** by reaction with chloroacetonitrile **13**. (B) The formation of treprostinil *N*-acyl methylsulfonamide **5** required at least 2 equiv of sodium hydride for bromoacetyl acylmethylsulfonamide **14** to undergo reaction with triol **12**. (C) The treprostinil triethylene glycol ester analogue **16** was synthesised from the ethylene glycol bromoacetyl bromide **15** and the triol **12** in mild basic conditions. Isolated yields are shown.

Hydrolysis of the ethyl ester **8** was conducted using LiOH in THF/methanol/water to give the bis-benzyl treprostinil **9** (89%) needed for coupling with methanesulfonamide **6**. Coupling was achieved using two different reagents reported in the literature [37]. Firstly, carbonyldiimidazole (CDI) in the presence of DBU in THF at reflux gave the desired bis-benzyl treprostinil *N*-acyl methylsulfonamide **10** (NMR, MS) in 21% yield after column chromatography. Secondly, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) and dimethylaminopyridine (DMAP) in dichloromethane (DCM) at room temperature [38]

Please cite this article as: C. Picken, P. Laing, L. Shen et al., Synthetic routes to treprostinil *N*-acyl methylsulfonamide, Tetrahedron Letters, https://doi.org/10.1016/j.tetlet.2019.151428

afforded the desired product in 46% yield following purification by column chromatography. Benzyl deprotection gave treprostinil *N*-acyl methylsulfonamide **5** (64%).

Although the overall yield could be improved by direct benzylation, the majority of losses occurred during the coupling of treprostinil **5** to methane sulfonamide **6**. Since treprostinil is an expensive starting material, the cumulative losses due to protection and deprotection steps, and the low efficiency of the coupling reactions of methanesulfonamide **6**, are not optimal for preparing treprostinil *N*-acyl methylsulfonamide **5**. The triol precursor **12** underwent selective alkylation at the aryl hydroxy with chloroacetonitrile **13** followed by hydrolysis to give treprostinil **2** (Scheme 3A) [39].

To avoid the aforementioned limitations, we found that it was possible to prepare treprostinil *N*-acyl methylsulfonamide **5** in 20% isolated yield after purification by the alkylation of the triol precursor **12** with the bromo-sulfonamide adduct **14** using excess NaH (Scheme 3B) [40]. Excess NaH was required due to the acidic proton in the bromo-sulfonamide **14** to give the desired treprostinil *N*-acyl methylsulfonamide **5**. The product structure was confirmed by ^{1}H NMR spectroscopy (Fig. S1). Alkylation of the triol precursor **12** with an α-bromo ester (e.g. ester **15**, Scheme 3C) that does not have an acidic proton adjacent to the carbonyl, as does bromo-sulfonamide **14**, is easily accomplished in good yield (>80%) using potassium carbonate in acetone at reflux as described by Kokotos and co-workers [41].

The overall yield to prepare treprostinil *N*-acyl methylsulfonamide **5** directly from the triol precursor **12** is similar to the protecting group strategies starting from treprostinil **2** because deprotonation of the acidic proton in bromoacetyl acylmethylsulfonamide **14** reduces the electrophilicity for alkylation to the triol **12**. The treprostinil triol precursor **12** is useful however for the preparation of a variety of prodrug candidates using a wide range of α-halo derivatives (e.g. ethylene glycol bromoacetyl bromide **15**).

The biological effects of IP agonists can be evaluated by the conversion of ATP to cyclic AMP in stable cells expressing the IP receptor [4,42]. *In vivo*, an increase in intracellular cyclic AMP results in a vasodilatory, anti-proliferative and anti-thrombotic response [9]. The concentration-dependent response of treprostinil **2** and treprostinil *N*-acyl methylsulfonamide **5** was evaluated over the concentration range of 0.01–1000 nM in HEK-293 cells expressing the human IP receptor. The log concentration causing 50% of the maximal response (log EC_{50}) for cyclic AMP generation was 30-fold lower for treprostinil *N*-acyl methylsulfonamide **5** compared to treprostinil **2** (Fig. 4). This EC_{50} value for treprostinil was similar to that calculated previously in the same cell line [42]. The 30-fold difference in the EC_{50} of treprostinil *N*-acyl methylsulfonamide **5** and treprostinil **2** is double that of selexipag and ACT-333679 (13-fold difference) as measured in a similar cyclic AMP assay [27].

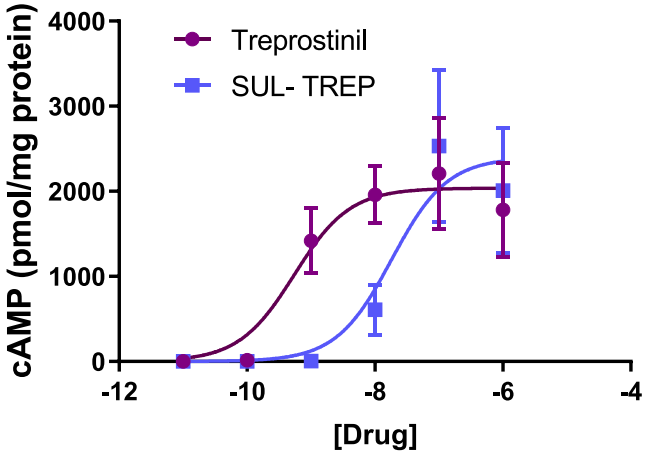


Fig. 4. Concentration–response relationship of intracellular cyclic AMP changes induced by treprostinil **2** and treprostinil *N*-acyl methylsulfonamide **5** in HEK-293 cells stably transfected with the human IP receptor. Cyclic-AMP concentration (mean ± SEM; n = 4) was measured following drug treatment (15 min) and normalised to cell protein content. Data points were fit with a unity sigmoidal-curve using Prism software. The concentration giving a half-maximal response (EC_{50}) for treprostinil **2** and treprostinil *N*-acyl methylsulfonamide **5** (SUL-TREP) was 0.59 nM and 18 nM, respectively.

Employment of a stable prodrug with reduced activity for the parenteral administration of treprostinil is hypothesised to avoid premature drug release which is predicted to translate clinically to a reduction in side effects experienced upon administration. With a favourable stability profile, a prodrug strategy may also enable a depot administration rather than continuous infusion. Moreover, treprostinil *N*-acyl methylsulfonamide **5** (like selexipag **3**) may be amenable to oral dosing, with concomitant advantages of therapeutic ratio and convenience of administration. These features remain to be established in further research. The encouraging results presented indicate that *in vivo* studies are warranted.

Acknowledgements

We are grateful to the Biotechnology and Biological Science Research Council (BBSRC grant number BB/L015803/1) and Lung Biotechnology PBC (Silver Spring, MD, USA) for funding this project and the CASE PhD studentship for CP. We are also grateful to United Therapeutics Corporation (Research Triangle Park, NC, USA) for supplying samples of the triol intermediate **12** and treprostinil **2**.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.151428.

References

[1] R.T. Schermuly, H.A. Ghofrani, M.R. Wilkins, F. Grimminger, Nat. Rev. Cardiol. 8 (2011) 443–455.
[2] G. Simonneau, N. Galiè, L.J. Rubin, D. Langleben, W. Seeger, G. Domenighetti, S. Gibbs, D. Lebrec, R. Speich, M. Beghetti, S. Rich, A. Fishman, J. Am. Coll. Cardiol. 43 (2004) 5S–12S.
[3] R. Del Pozo, I. Hernandez Gonzalez, P. Escribano-Subias, Expert Rev. Respir. Med. 11 (2017) 491–503.
[4] E. Falcetti, S.M. Hall, P.G. Phillips, J. Patel, N.W. Morrell, S.G. Haworth, L.H. Clapp, Am. J. Respir. Crit. Care Med. 182 (2010) 1161–1170.
[5] L.J. Rubin, J. Mendoza, M. Hood, M. McGoon, R. Barst, W.B. Williams, J.H. Diehl, J. Crow, W. Long, Ann. Intern Med. 112 (1990) 485.
[6] L.J. Rubin, B.M. Groves, J.T. Reeves, M. Frosolono, F. Handel, A.E. Cato, Circulation 66 (1982) 334–338.
[7] R.J. Barst, L.J. Rubin, W.A. Long, M.D. McGoon, S. Rich, D.B. Badesch, B.M. Groves, V.F. Tapson, R.C. Bourge, B.H. Brundage, S.K. Koerner, D. Langleben, C.A. Keller, S. Murali, B.F. Uretsky, L.M. Clayton, M.M. Jöbsis, S.D. Blackburn, D. Shortino, J.W. Crow, N. Engl. J. Med. 334 (1996) 296–301.
[8] L.H. Clapp, J. Patel, Int. J. Respir. Care 6 (2010) 27–33.
[9] L.H. Clapp, R. Gurung, Prostaglandins Other Lipid Mediat. 120 (2015) 56–71.
[10] M. Humbert, N.W. Morrell, S.L. Archer, K.R. Stenmark, M.R. MacLean, I.M. Lang, B.W. Christman, E.K. Weir, O. Eickelberg, N.F. Voelkel, M. Rabinovitch, J. Am. Coll. Cardiol. 43 (2004) 13S–24S.
[11] F. Torres, L.J. Rubin, Expert Rev. Cardiovasc. Ther. 11 (2013) 13–25.
[12] V.F. Tapson, C.J. Sanchez Diaz, G.M. Bohns Meyer, T. Pulido, P. Sepulveda, K.Y. Wang, C.Q. Deng, R. Grover, D. Solum, A. Ousmanou, R.J. White, J. Heart Lung Transplant. 38 (2019) S94–S95.
[13] M.S. Kingman, S. Lombardi, J. Infus. Nurs. 37 (2014) 442–451.
[14] K. Laliberte, C. Arneson, R. Jeffs, T. Hunt, M. Wade, J. Cardiovasc. Pharmacol. 44 (2004) 209–214.
[15] C. Picken, K.C. Fragkos, M. Eddama, G. Coghlan, L.H. Clapp, C. Picken, K.C. Fragkos, M. Eddama, G. Coghlan, L.H. Clapp, J. Clin. Med. 8 (2019) 481.
[16] K. Kabashima, M. Nagamachi, T. Honda, C. Nishigori, Y. Miyachi, Y. Tokura, S. Narumiya, Lab Investig. 87 (2007) 49–55.
[17] H. Hohjoh, T. Inazumi, S. Tsuchiya, Y. Sugimoto, Biochimie 107 (2014) 78–81.
[18] B.J.R. Whittle, S. Moncada, Prog. Med. Chem. 21 (1985) 237–279.

[19] F. Leifer, D. Omiatek, V. Malinin, J. Ong, Z. Li, P. Klecha, R. Chapman, D. Salvail, C. Laurent, W. Perkins, Eur. Respir. J. 44 (2014) P2356-.
[20] United Therapeutics, United Therapeutics, NIH Clin. https://clinicaltrials.gov/ct2/show/NCT02149095 (accessed April 20, 2018).
[21] M.R. Corboz, Z. Li, V. Malinin, A.J. Plaunt, D.M. Konicek, F.G. Leifer, K.-J. Chen, C. E. Laurent, H. Yin, M.C. Biernat, D. Salvail, J. Zhuang, F. Xu, A. Curran, W.R. Perkins, R.W. Chapman, J. Pharmacol. Exp. Ther. 363 (2017) 348–357.
[22] D. Han, C. Fernandez, E. Sullivan, D. Xu, W. Perkins, M. Darwish, C. Rubino, in: 4.3 Pulmonary Circulation and Pulmonary Vascular Diseases, vol. 48, European Respiratory Society, PA 2403, 2016.
[23] R.W. Chapman, V. Malinin, D. Konicek, F. Leifer, Z. Li, J. Zhuang, F. Xu, W.R. Perkins, in: American Thoracic Society International Conference Meetings Abstracts, Denver, 2017, A52.
[24] Ascendis Pharma A/S, Ascendis Pharma A/S, Press Release. https://www.prnewswire.com/news-releases/ascendis-pharma-as-announces-results-of-phase-1-study-of-transcon-treprostinil-300073527.html (accessed April 20, 2018).
[25] European Medicines Agency, Uptravi (selexipag): Summary of Product Characteristics, London, 2016.
[26] T. Asaki, K. Kuwano, K. Morrison, J. Gatfield, T. Hamamoto, M. Clozel, J. Med. Chem. 58 (2015) 7128–7137.
[27] K. Kuwano, A. Hashino, T. Asaki, T. Hamamoto, T. Yamada, K. Okubo, K. Kuwabara, J. Pharmacol. Exp. Ther. 322 (2007) 1181–1188.
[28] A. Nakamura, T. Yamada, T. Asaki, Bioorg. Med. Chem. 15 (2007) 7720–7725.
[29] A. Ammazzalorso, B. De Filippis, L. Giampietro, R. Amoroso, Chem. Biol. Drug Des. 90 (2017) 1094–1105.
[30] G. Simonneau, A. Torbicki, M.M. Hoeper, M. Delcroix, K. Karlócai, N. Galiè, B. Degano, D. Bonderman, M. Kurzyna, M. Efficace, R. Giorgino, I.M. Lang, Eur. Respir. J. 40 (2012) 874–880.
[31] S. Huang, P.J. Connolly, R. Lin, S. Emanuel, S.A. Middleton, Bioorg. Med. Chem. Lett. 16 (2006) 3639–3641.
[32] R. Shorr, M. Rothblatt, M. Bentley, D. Michael, X. Zhao, PCT/US2000/008240, 2000.
[33] U. Hershel, H. Rau, T. Lessman, N. Bisek, G. Maitro, K. Sprogoe, T. Wegge, O. Kiel, J. Zettler, 2013024052, 2013.
[34] V. Malinin, W. Perkin, F. Leifer, D. Konicek, Z. Li, Z. Plaunt, WO2015061720, 2015.
[35] K. Sprogoe, H. Rau, U. Hersel, T. Wegge, O. Keil, J. Zettler, 2013024051, 2013.
[36] S.S. Lopez, G.B. Dudley, Beilstein J. Org. Chem. 4 (2008) 44.
[37] K. Rikimaru, T. Wakabayashi, H. Abe, T. Tawaraishi, H. Imoto, J. Yonemori, H. Hirose, K. Murase, T. Matsuo, M. Matsumoto, C. Nomura, H. Tsuge, N. Arimura, K. Kawakami, J. Sakamoto, M. Funami, C.D. Mol, G.P. Snell, K.A. Bragstad, B.-C. Sang, D.R. Dougan, T. Tanaka, N. Katayama, Y. Horiguchi, Y. Momose, Bioorg. Med. Chem. 20 (2012) 3332–3358.
[38] M.M. Lorion, F.J.S. Duarte, M.J. Calhorda, J. Oble, G. Poli, Org. Lett. 18 (2016) 1020–1023.
[39] R.M. Moriarty, N. Rani, L.A. Enache, M.S. Rao, H. Batra, L. Guo, R.A. Penmasta, J. P. Staszewski, S.M. Tuladhar, O. Prakash, D. Crich, A. Hirtopeanu, R. Gilardi, J. Org. Chem. 69 (2004) 1890–1902.
[40] R.C. Oslund, N. Cermak, M.H. Gelb, J. Med. Chem. 51 (2008) 4708–4714.
[41] G. Kokotos, A.J. Feuerherm, E. Barbayianni, I. Shah, M. Sæther, V. Magrioti, T. Nguyen, V. Constantinou-Kokotou, E.A. Dennis, B. Johansen, J. Med. Chem. 57 (2014) 7523–7535.
[42] B.J. Whittle, A.M. Silverstein, D.M. Mottola, L.H. Clapp, Biochem. Pharmacol. 84 (2012) 68–75.

Please cite this article as: C. Picken, P. Laing, L. Shen et al., Synthetic routes to treprostinil *N*-acyl methylsulfonamide, Tetrahedron Letters, https://doi.org/10.1016/j.tetlet.2019.151428