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An optimized and versatile synthesis to pyridinylimidazole-type p38α mitogen activated protein kinase inhibitors†

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An optimized strategy for the synthesis of the potent p38α mitogen-activated protein kinase inhibitors 2-(2-hydroxyethylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (**3**) and 2-(2,3-dihydroxypropylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (**4**) starting from 2-fluoro-4-methylpyridine is reported. In contrast to a previously published synthesis starting from 2-bromo-4-methylpyridine, the overall yield could be increased from 3.6% to 29.4%. Moreover, this strategy avoids the use of palladium as a catalyst and is more diverse and versatile. Using this optimized protocol, both enantiomers of potent inhibitor **3** were synthesized. Biological data demonstrated that the (*S*)-enantiomer is the two times more potent eutomer.

Introduction

The p38α mitogen-activated protein (MAP) kinase is a serine/threonine kinase, which links extracellular signals to the intracellular machinery modulating a plethora of cellular processes, *e.g.*, the release of pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-1β.[1] Therefore, the inhibition of p38α MAP kinase was evaluated as a therapeutic strategy for the treatment of cytokine-driven diseases like rheumatoid arthritis or psoriasis. Other studies suggest an important role of p38α MAP kinase in the pathogenesis of neurodegenerative diseases like Parkinson's disease, Alzheimer's disease or multiple sclerosis.[2–4] Recently, several clinical trials investigating inhibitors of p38α MAP kinase for the treatment of chronic obstructive pulmonary disease were terminated.[5,6] Trisubstituted pyridinylimidazoles, like the prototypical inhibitor SB203580 (**1**) or ML3403 (**2**), are amongst the most prominent adenosine triphosphate (ATP) competitive inhibitors of p38α MAP kinase (Fig. 1).[7–9]

In 2008, we reported 2-(2-hydroxyethylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (**3**) and 2-(2,3-dihydroxypropylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (**4**) as optimized p38α MAP kinase inhibitors derived from ML3403 (Fig. 1).[10] The hydroxyl containing moie-

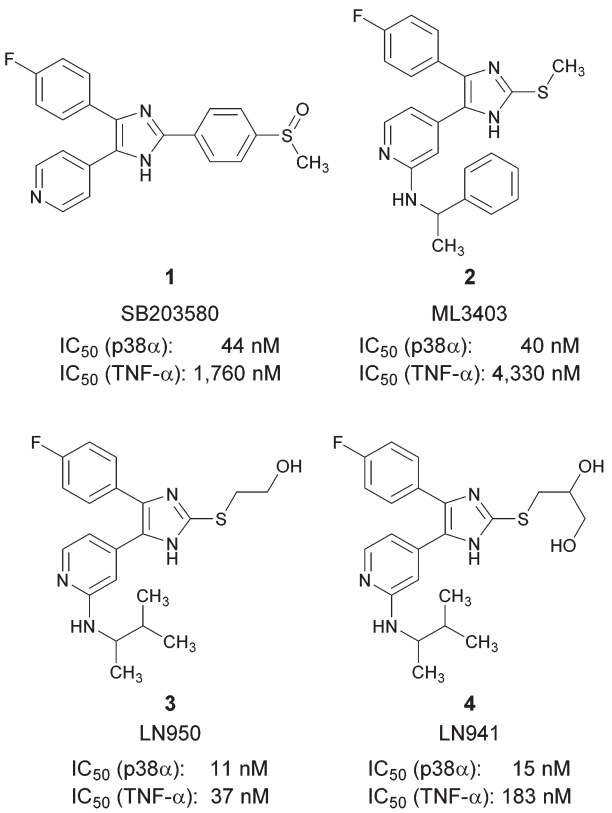

Fig. 1 Pyridinylimidazole based p38α MAP kinase inhibitors.

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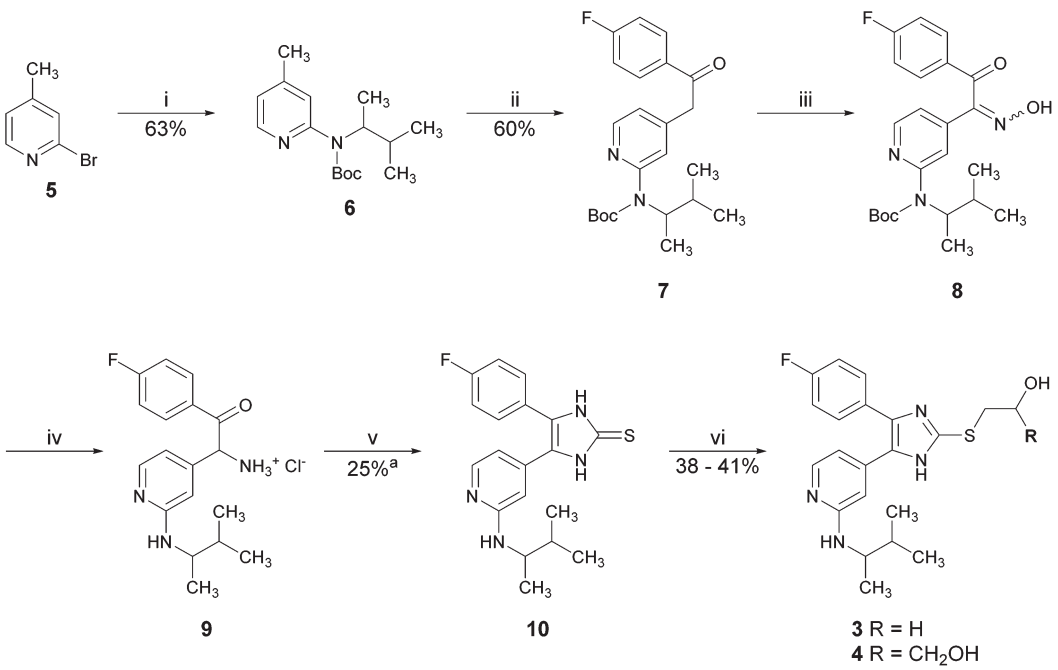
ties at the imidazole C^2-position may interact with the ribose and the phosphate binding site of the enzyme. In contrast to the parent compound ML3403, compounds **3** and **4** show improved p38α MAP kinase inhibitory activity and a strong increase in inhibition of LPS-stimulated TNF-α release from human whole blood (in case of **3**, >110-fold increase compared to **2**). In order to profile these inhibitors in further *in vitro* and *in vivo* experiments, a high yielding and scalable synthesis is required.

The previously reported synthetic route toward inhibitors **3** and **4** starts from 2-bromo-4-methylpyridine (**5**) and is depicted in Scheme 1.[10,11] In the first step of this synthesis, the amino moiety was introduced at the pyridine C^2-position *via* Buchwald–Hartwig arylamination and the obtained secondary amine was directly protected with the Boc-group. Picoline **6** was converted into the ethanone **7** by reaction with ethyl 4-fluorobenzoate using NaHMDS as a base. Upon treatment with an excess of sodium nitrite in acetic acid, ethanone **7** was converted into the α-hydroxyiminoketone **8**. Reduction of oxime **8** to a corresponding amine hydrochloride **9** accompanied by deprotection of the Boc-group was achieved by hydrogenation in methanolic hydrogen chloride using Pd/C as a catalyst. Cyclization was accomplished by treatment with potassium thiocyanate. Finally, the thione **10** was converted into the target compounds **3** and **4** by nucleophilic substitution reactions with 2-bromoethanol and 3-bromopropane-1,2-diol, respectively. The total yield of this synthetic strategy to pyridinylimidazoles **3** and **4** starting from 2-bromo-4-methylpyridine (**5**) is 3.6% and 3.9% (7 linear steps), respectively.

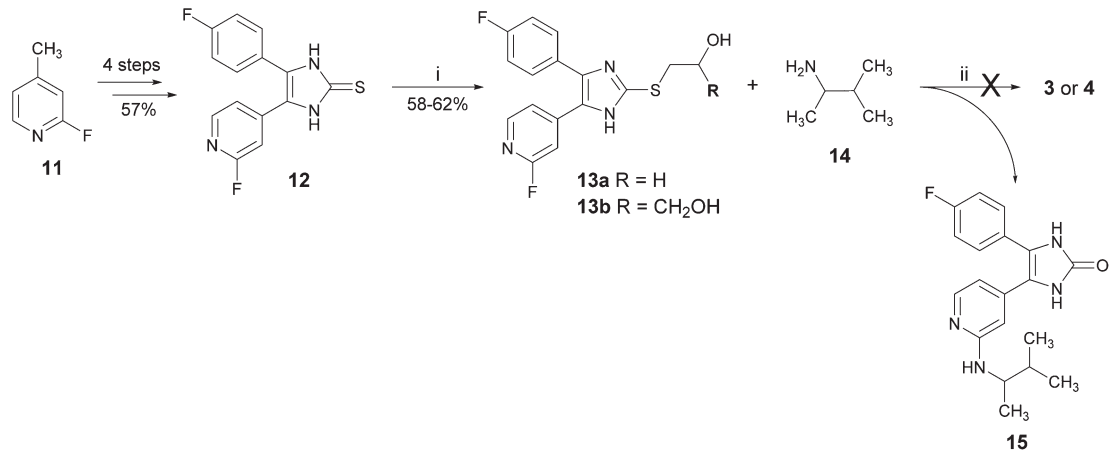
The major limitations of this synthetic strategy for scale-up are the introduction of the amino moiety in the first step of the synthesis, which requires the addition and removal of a protecting group and its low overall yield (>4%). Moreover, the required early stage use of the palladium catalyst is quite cost intensive due to the higher amount needed in scale-up.

Another attempted synthetic strategy to the target compounds **3** and **4** reported from our group is depicted in Scheme 2.[12] The introduction of the 3-methyl-2-butylamino moiety at the pyridine C^2-position as well as the moiety at the imidazole C^2–S-position was envisaged in the last two steps of the synthesis. The key compound for this route 4-(4-fluorophenyl)-5-(2-fluoropyridin-4-yl)-1,3-dihydroimidazole-2-thione (**12**) was prepared according to a protocol from Laufer and coworkers[8,13] in four steps starting from 2-fluoro-4-methylpyridine (**11**) in an overall yield of 57% (for details, see Scheme S1 in the ESI†). The alkylsulfanyl moiety was introduced by nucleophilic substitution of thione **12** and the appropriate alkyl halide. In the last step, the fluorine atom in **13a** and **13b** should be displaced by 3-methyl-2-butylamine (**14**) to obtain **3** and **4**, respectively. By heating **13a** or **13b** with the amine **14**, we observed an unexpected transformation of 2-alkylsulfanylimidazoles bearing a 2-hydroxyethyl or a 2,3-dihydroxypropyl moiety at the imidazole C^2–S-position into the imidazol-2-one derivative **15**.

Herein, we report a distinct and optimized procedure to prepare pyridinylimidazoles **3** and **4** in higher yields and with increased versatility compared to the previously published



Scheme 1 Reported synthesis of pyridinylimidazoles **3** and **4**. Reagents and conditions: (i) 1. 3-methyl-2-butylamine (rac.), $Pd_2(dba)_3$, *t*-BuONa, BINAP, toluene, reflux temperature; 2. Boc_2O, DMAP, CH_2Cl_2, r.t.; (ii) NaHMDS, ethyl 4-fluorobenzoate, THF, 0 °C – r.t.; (iii) $NaNO_2$, acetic acid, 10 °C – r.t.; (iv) Pd/C 10%, MeOH/HCl, H_2, atmospheric pressure, r.t.; (v) KSCN, DMF, reflux temperature, 3 h; (vi) 2-bromoethanol, *t*-BuOK, reflux temperature, 1.5 h in case of compound **3** or 3-bromopropane-1,2-diol, *t*-BuOK, reflux temperature, 3 h in case of compound **4**; [a]yield over three steps, compounds **8** and **9** were not isolated.



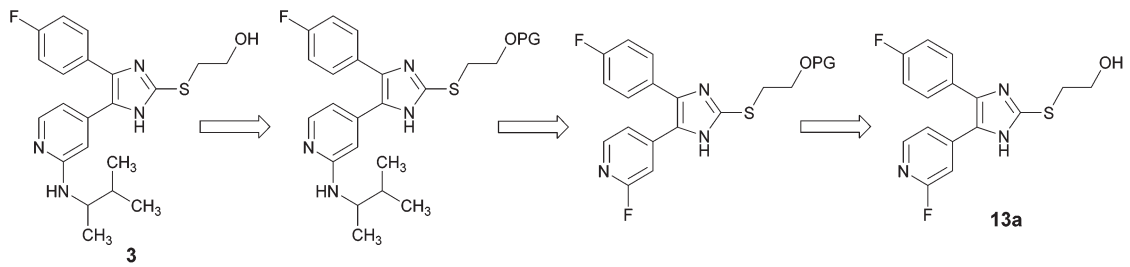
Scheme 2 Unexpected reaction of 2-alkylimidazoles **13a** and **13b** into imidazol-2-ones. Reagents and conditions: (i) EtONa, methanol, 2-bromoethanol, r.t., 4 h (for synthesis of **13a**) or EtONa, methanol, 3-bromopropane-1,2-diol, r.t., 16 h (for synthesis of **13b**); (ii) 160 °C, 10 h, sealed tube.

protocols. Moreover, the described synthetic strategy avoids the use of palladium as a catalyst for introduction of the amino moiety at the pyridine C^2-position.

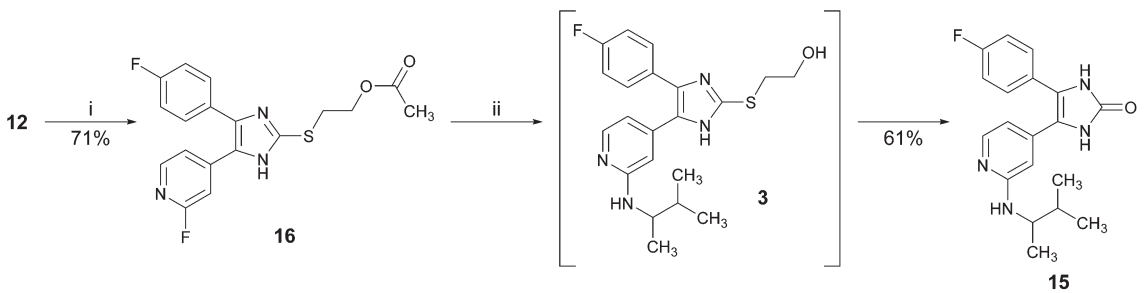
Results & discussions

In order to avoid the aforementioned conversion of 2-alkylsulfanylimidazoles bearing a 2-hydroxyethyl or a 2,3-dihydroxypropyl moiety at the imidazole C^2–S-position into the corresponding imidazol-2-one derivatives, we pursued the strategic introduction of a hydroxyl protecting group at the imidazole C^2–S moiety prior to this step and its cleavage in the subsequent step (Scheme 3).

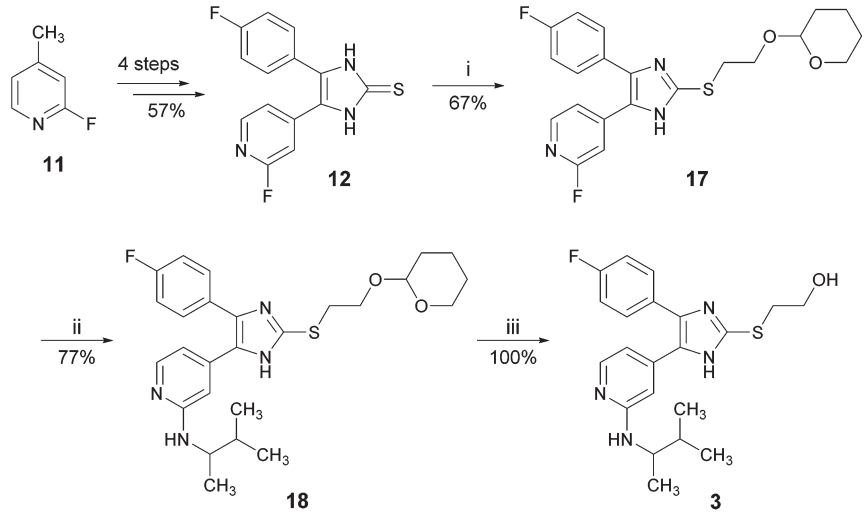
Initially, we attempted to make use of an acetyl ester to protect the hydroxy function (Scheme 4).[14] Therefore, imidazole-2-thione **12** was treated with 2-bromoethyl acetate under mildly basic conditions in order to obtain ester **16**, bearing the acetyl protected hydroxyethyl moiety at the imidazole C^2–S-position. Compound **16** was reacted with 3-methyl-2-butylamine in a nucleophilic aromatic substitution reaction. Under these harsh and basic conditions (160 °C, excess of amine), the acetyl protecting group was cleaved and imidazol-2-one **15** was isolated as the major product of this reaction. Decrease of



Scheme 3 Retrosynthetic approach for synthesis of **3** (PG, protecting group).



Scheme 4 Conversion of ester **16** to imidazol-2-one **15**. Reagents and conditions: (i) 2-bromoethyl acetate, Cs_2CO_3, DMF, 55 °C, 30 min; (ii) 3-methyl-2-butylamine (rac.), 160 °C, 16 h.



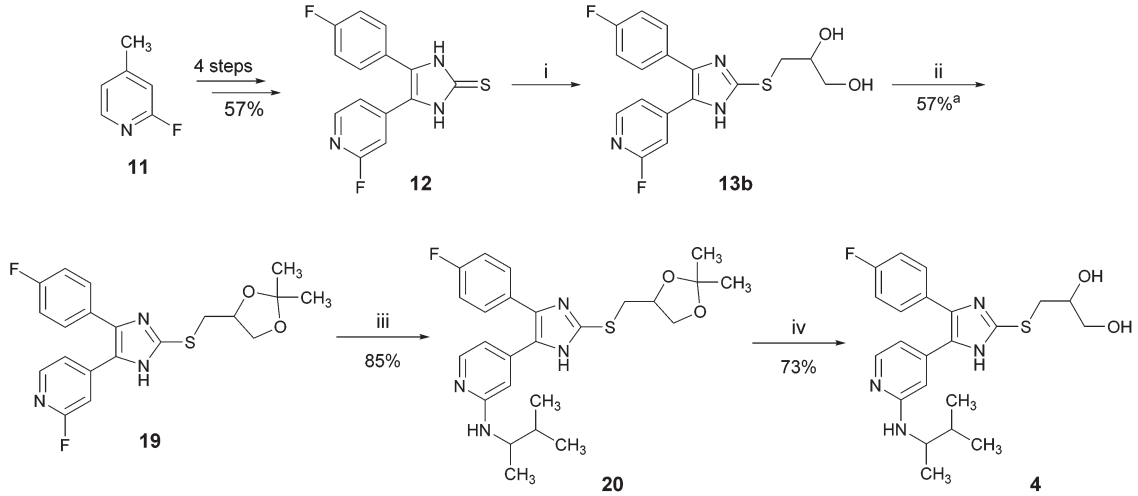
Scheme 5 Optimized synthesis of **3** using THP as the hydroxyl protecting group. Reagents and conditions: (i) *t*-BuONa, methanol, 2-(2-bromoethoxy)-tetrahydro-2*H*-pyran, 30 min, 50 °C; (ii) 3-methyl-2-butylamine (rac.), 160 °C, 16 h, sealed tube; (iii) 1.25 M HCl/EtOH, 3 h, r.t.

both, reaction temperature and excess of amine resulted in a slow or no conversion.

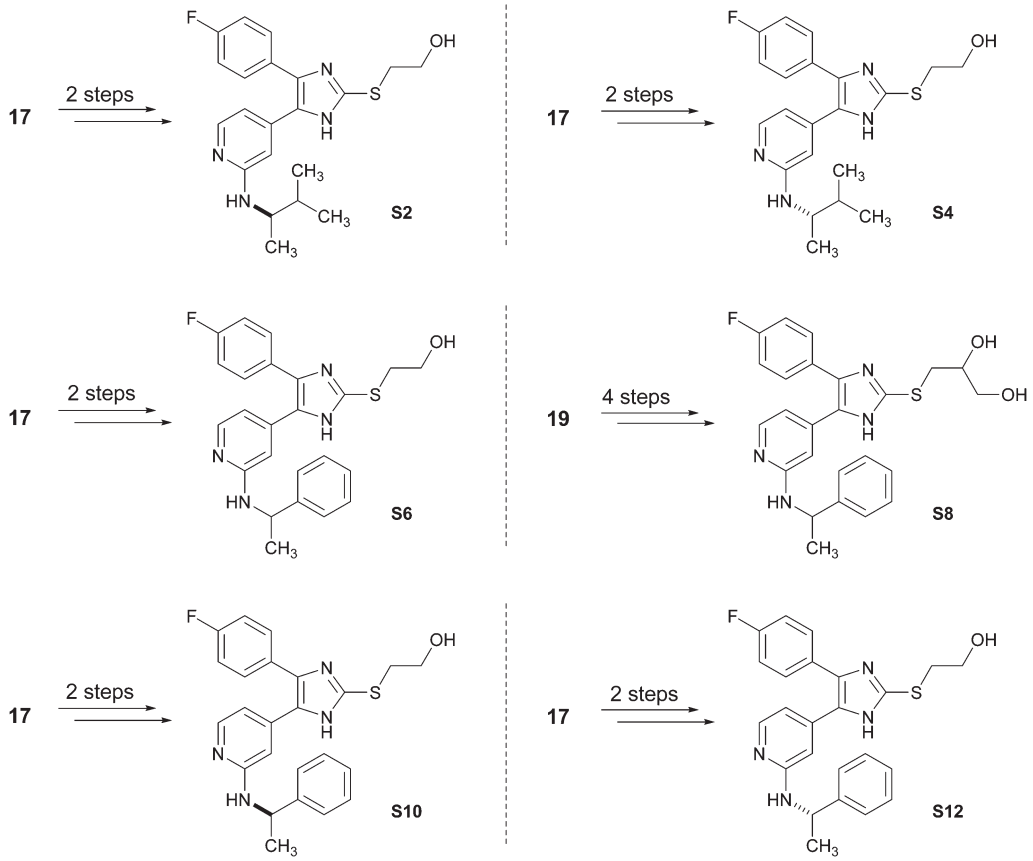
As another protecting group for the hydroxy function, we used the acid labile but base stable tetrahydropyranyl (THP) group (Scheme 5).[15] Imidazole-2-thione **12** was reacted with 2-(2-bromoethoxy)-tetrahydro-2*H*-pyran to give compound **17** in good to moderate yields. In the next step, the amino function at the pyridine C^2-position was introduced *via* nucleophilic aromatic substitution under the above mentioned conditions. To our delight, we found the THP-group to be sufficiently stable and compound **18**, the THP-protected derivative of **3**, was obtained in good yields. Finally, treatment of **18** with ethanolic hydrochloric acid solution resulted in cleavage of the THP group and the target compound **3** was obtained in quantitative yields.

With a total yield of 29.4% in 7 linear steps, the optimized synthesis of compound **3** starting from 2-fluoro-4-methylpyridine (**11**) compares very well to the reported synthesis starting from 2-bromo-4-methylpyridine (**5**), giving more than eight times higher yields (compare Scheme 5 *vs.* Scheme 1). Using this protocol, 2.3 g of inhibitor **3** were synthesized starting from 3.0 g imidazole-2-thione **12** in similar yields as reported in Scheme 5 (for details, see ESI†).

For optimizing the synthesis of inhibitor **4**, we used an acetal group to protect the 1,2-dihydroxy function.[16] Acetals are generally stable under basic conditions and liberate the diol under acidic conditions. The optimized route to **4** is depicted in Scheme 6. The dihydroxypropylsulfanylimidazole **13b** was converted into the corresponding acetonide **19** by



Scheme 6 Optimized synthesis of **4** using acetal as the 1,2-dihydroxyl protecting group. Reagents and conditions: (i) *t*-BuONa, methanol, 3-bromopropane-1,2-diol, 70 °C, 1 h; (ii) acetone, *p*-TsOH, reflux temperature, 24 h; (iii) 3-methyl-2-butylamine (rac.), 160 °C, 16 h, sealed tube; (iv) 2 M aq. HCl, r.t., 3 h; [a]yield over two steps, compound **13b** was not isolated.



Scheme 7 Synthesis of pyridinylimidazoles **S2**, **S4**, **S6**, **S8**, **S10** and **S12**. For reagents and conditions, see the ESI.†

treatment with acetone under *p*-toluenesulfonic acid (*p*-TsOH) catalysis. In the next step, the amino function was introduced at the pyridine C^2-position *via* nucleophilic aromatic substitution and finally the acetal protecting group was cleaved with diluted hydrochloric acid to yield inhibitor **4**. The overall yield of this protocol starting from **11** is 20.2% (8 linear steps), which is five times higher than the previously reported synthesis starting from **5** (Scheme 1, total yield: 3.9%).

Both optimized synthetic strategies presented in Schemes 5 and 6 are versatile and diverse since the introduction of both, the imidazole C^2–S moiety and the pyridine C^2–amino function are in the last steps of the sequence. Therefore, these strategies allow a facile and high-yielding access to further pyridinylimidazole derivatives (Scheme 7, Schemes S2–S7, ESI†). Starting from intermediates **17** or **19**, both enantiomers of **3** (compounds **S2** and **S4**), the hydroxyethylsulfanyl and dihydroxypropylsulfanyl derivatives of ML3403 (**S6** and **S8**) and both enantiomers of **S6** (compounds **S10** and **S12**) were prepared. The synthesized pyridinylimidazoles were tested in an ELISA-assay for their ability to inhibit the p38α MAP kinase (Table 1).[17] Furthermore, the potential to inhibit the LPS-stimulated TNF-α release from human whole blood was determined for **S2** and **S4**, the most promising inhibitors in this set, and compared to the racemic mixture, compound **3**. In case of the chiral inhibitors **S2**, **S4**, **S10** and **S12**, the biological data revealed

Table 1 Biological data for selected pyridinylimidazoles

	IC_{50} ± SEM	
Cmpd	p38α MAP kinase[a]	TNF-α release[b]
3 (LN950)	11 ± 1 nM[c]	37 ± 4 nM[c]
4 (LN941)	15 ± 2 nM[c]	183 ± 16 nM[c]
S2 [(*R*)-**3**]	19 ± 2 nM	84 ± 3 nM
S4 [(*S*)-**3**]	14 ± 2 nM	20 ± 3 nM
S6	32 ± 3 nM	nd[d]
S8	41 ± 7 nM[c]	936 ± 15 nM[c]
S10 [(*R*)-**6**]	42 ± 1 nM	nd
S12 [(*S*)-**6**]	22 ± 0.2 nM	nd

[a] Results are from three experiments. [b] Tests were carried out in duplicate. [c] Test data are from Koch *et al.*[10] [d] nd. not determined.

the (*S*)-enantiomer as the eutomer. Compared to the racemic inhibitor **3**, the LPS-stimulated TNF-α-release is inhibited two times more by the (*S*)-enantiomer **S4**, whereas, the (*R*)-enantiomer **S2** is as expected two times less active in this assay.

Conclusion

An optimized and diverse synthetic approach for the preparation of pyridinylimidazole-based p38α MAP kinase inhibitors

is described. Compared to a previously reported method, an up to eight times higher overall yield was obtained.

A series of eight pyridinylimidazoles was synthesized using these optimized conditions. In case of the chiral inhibitor **3**, biological evaluation showed that the (*S*)-enantiomer is the two times more potent eutomer.

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