Expedient Synthesis of 1,3-Substituted Benzene Peptidomimetics

Anders Bach, Kristian Strømgaard*

Department of Medicinal Chemistry, The Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark
Fax +4535336041; E-mail: krst@farma.ku.dk

Received 8 December 2010; revised 5 January 2011

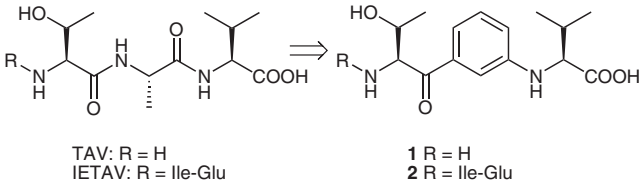
Abstract: A synthetic route for replacing the central amino acid in the tripeptide Thr-Ala-Val (TAV) with a 1,3-substituted benzene ring was developed. L-Threonine was introduced into the benzene ring by a Grignard reaction with protected L-threoninal, where the nature of the side-chain protecting group was found to be of utmost importance. Subsequently, L-valine was introduced by a copper-mediated amination. Overall, the tripeptide analogue was obtained in six steps with an overall yield of 18%. An orthogonal protecting group strategy allowed attachment of the tripeptide analogue to a solid support and subsequent preparation of the corresponding pentapeptide analogue. Both compounds were tested in a plasma stability assay, showing improved stability compared to their peptide counterparts.

Key words: amino acids, peptides, Grignard reaction, nucleophilic aromatic substitution, copper

Converting bioactive peptides into rigid analogues provides a general and promising strategy for developing drug-like inhibitors of protein–protein interactions, peptide–protein interactions or proteolytic enzymes. Several of these interactions are mediated by peptide β-strands, a highly abundant structural motif in proteins made by a continuous stretch of amino acids adopting an extended conformation.[1,2] Constrained scaffolds that are able to mimic the topographical and pharmacophoric features of the β-strand will theoretically display increased affinity, as the entropy penalty is reduced, as well as higher selectivity towards the protein target.[1,3] Also, by constraining the structure or replacing amide bonds reduced susceptibility towards proteolysis is obtained.[4]

In this paper, we explore synthetic routes to peptidomimetic **1**, where the central amino acid of the tripeptide Thr-Ala-Val (TAV), an inhibitor of PDZ (postsynaptic density protein-95/discs large/zonula occludens-1) domain mediated interactions,[5] is replaced with a 1,3-substituted benzene ring (Scheme 1). This design provides a rigid, extended conformation of the two flanking amino acids, threonine and valine, while the overall proportions and geometry of **1** are similar to the tripeptide. Also, this design is applied to the pentapeptide IETAV, as in compound **2** (Scheme 1).

Compound **1** deviates from published benzene-based β-strand mimetics by the two amino acid moieties being connected to the aromatic ring via a carbonyl and an amino group, respectively, instead of amide bonds.[1,6] Also, compared to previously described pyridine-based β-strand mimetics,[1,7–9] compound **1** contains heteroatomic polar *and* chiral amino acid side chains and, in addition, free carboxylic acid and amino groups. Although these features, together with the *meta* position of the substituents, challenged the synthesis, we have developed a feasible and practical route for obtaining the target compounds, as described in the following.



TAV: R = H
IETAV: R = Ile-Glu

1 R = H
2 R = Ile-Glu

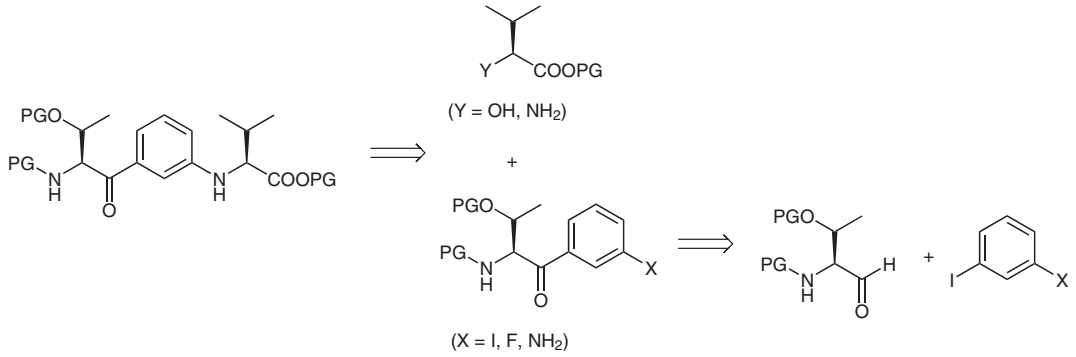
Scheme 1 Design of **1** and **2** as mimetics of TAV and IETAV

Based on a retrosynthetic analysis of **1** (Scheme 2), we hypothesized that the threonine moiety could be introduced by a Grignard coupling reaction of the corresponding protected aldehyde and subsequent oxidation,[9] while L-valine could either be incorporated by a Mitsunobu reaction, which would allow stereospecific alkylation of amines with the corresponding alcohol,[10] or by a direct amination with L-valine via a mild version of the Ullmann–Goldberg reaction.[11,12]

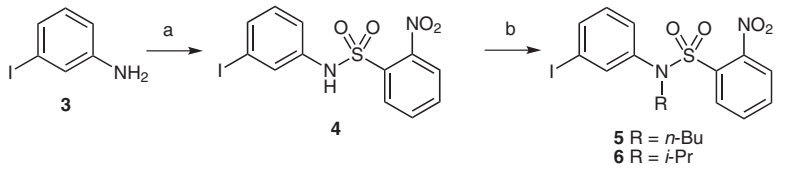
To probe the feasibility of the Mitsunobu reaction in this process, 3-iodoaniline (**3**) was applied as a model compound and readily reacted with *o*-nitrobenzenesulfonyl chloride (*o*-NBSCl) to furnish the nitrobenzenesulfonamide **4** (Scheme 3).[13] Compound **4** was reacted with butanol or isopropyl alcohol under Mitsunobu conditions, resulting in successful conversion (~90%) into the desired products, compounds **5** and **6**, respectively (Scheme 3).

The Grignard reaction was investigated by reacting **3** (1 equiv) with isopropylmagnesium chloride (2 equiv) and benzaldehyde (1 equiv) at various times and temperatures, but only starting material was observed. Increasing the relative amount of isopropylmagnesium chloride, using *tert*-butyllithium, or phenylmagnesium chloride followed by magnesium–halogen exchange with isopropylmagnesium chloride[14] also failed. It is most likely the electron-donating effect of the amino group and the absence of electron-withdrawing groups on the aromatic ring to compensate for this effect that lowers the reactivity of **3**.[14,15] Carrying out the Grignard reaction using either *o*-NBS-protected 3-iodoaniline **4** or the tetramethyldisilylazacy-

SYNTHESIS 2011, No. 5, pp 0807–0815
Advanced online publication: 08.02.2011
DOI: 10.1055/s-0030-1258425; Art ID: T52010SS



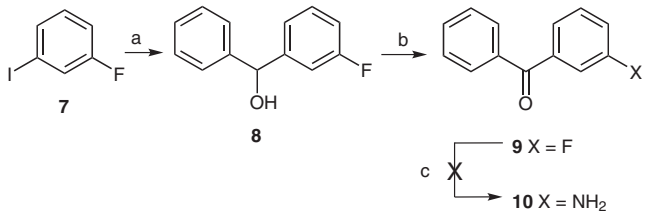
Scheme 2 Retrosynthetic analysis of target compound **1** (PG: protecting group)



Scheme 3 *Reagents and conditions*: (a) *o*-NBSCl, NaOAc, EtOH, H_2O, 80 °C, 30 min, 60%; (b) ROH, DIAD, Ph_3P, toluene, 80 °C, 2 h, **5**: 43%, **6**: 24%.

clopentane (STABASE) adduct of **3** (see Supporting Information),[16] also did not provide the desired product. Instead, 1-fluoro-3-iodobenzene (**7**) was used as a model system in the Grignard reaction, and reaction with benzaldehyde provided **8**, which followed by oxidation gave fluorobenzophenone **9** (Scheme 4).

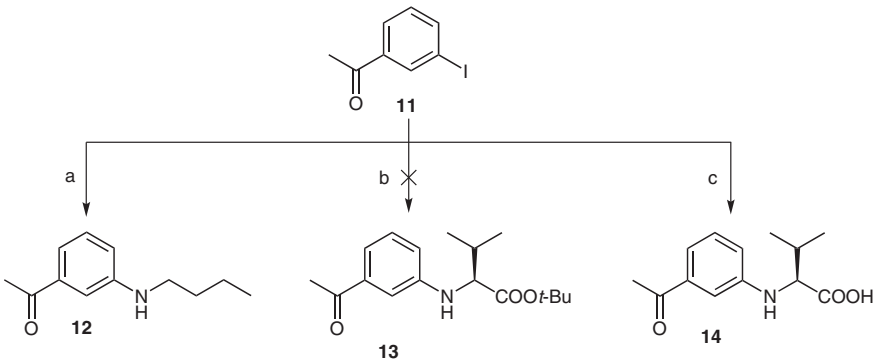
The subsequent nucleophilic aromatic substitution (S_NAr) reaction with aqueous ammonia under microwave conditions is feasible with 2-fluoropyridine as the starting material;[7] however, the same reaction with **9** was not successful in providing **10**. This low reactivity of **9** can be rationalized by the *meta* positioning of the electron-withdrawing carbonyl group relative to the fluorine and, in comparison, pyridine is generally more prone to S_NAr reactions because of efficient delocalization of electrons at the nitrogen atom.[8]



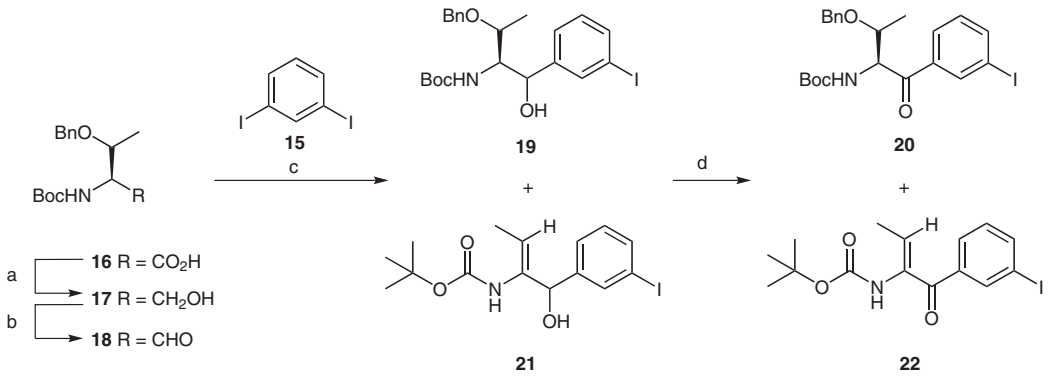
Scheme 4 *Reagents and conditions*: (a) PhCHO, *i*-PrMgCl, THF, 0 °C → r.t., 45 min, 86%; (b) DMP, CH_2Cl_2, r.t., 30 min, 88%; (c) 25% aq NH_3, 140 °C, microwave irradiation, 10–180 min.

Instead, copper(I) iodide/cesium acetate mediated amination was investigated as a means to introduce the valine moiety into the benzene scaffold. Initially, the feasibility of the reaction was demonstrated using 3′-iodoacetophenone (**11**) and butylamine,[12] which provided **12** as expected (Scheme 5). However, the use of *tert*-butyl-protected L-valine (H-Val-O-*t*-Bu) instead of butylamine did not provide **13**, but when unprotected L-valine and potassium carbonate were employed,[11] the desired product **14** was obtained.

The Grignard protocol was initially explored using 1,3-diiodobenzene (**15**) and benzaldehyde as model compounds, which furnished the monoalkylated compound, (3-iodophenyl)(phenyl)methanol, as expected (GCMS).[14] For the synthesis of compound **19**, *tert*-butoxycarbonyl-



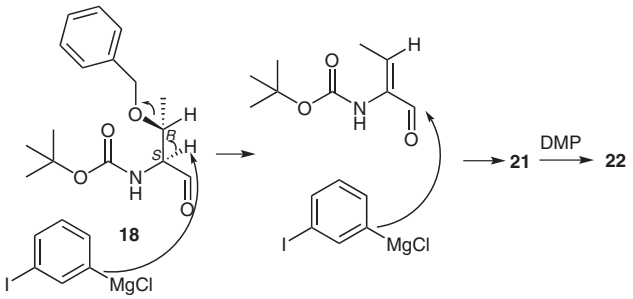
Scheme 5 *Reagents and conditions*: (a) $BuNH_2$, CuI, CsOAc, DMF, 90 °C, 5 h, 64%; (b) H-Val-O-*t*-Bu, CuI, CsOAc, DMF or DMSO, 90 °C, 48 h; (c) L-valine, CuI, K_2CO_3, DMF, 90 °C, 24 h, 47%.



Scheme 6 *Reagents and conditions*: (a) (i) isobutyl chloroformate, NMM, THF, –20 °C, 30 min; (ii) $NaBH_4$, MeOH, –20 °C, 1 h, 88%; (b) DMP, 0.1% H_2O in CH_2Cl_2, r.t., 15 min, 93%; (c) 1,3-diiodobenzene (**15**), *i*-PrMgCl, THF, –78 °C → r.t., 90 min; (d) DMP, 0.1% H_2O in CH_2Cl_2, r.t., 15 min, **20**: 27% from **18**.

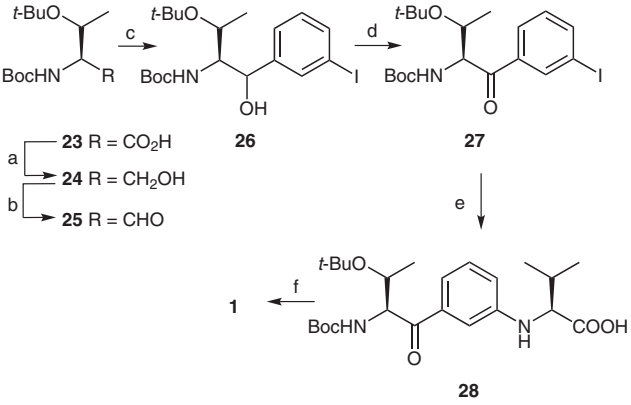
and benzyl-protected L-threoninal **18** was prepared, as previously described for other amino acids,[7,17] with the modification of adding a catalytic amount of water to accelerate the Dess–Martin periodinane (DMP) mediated oxidation (Scheme 6).[18]

The subsequent Grignard reaction with **18** (0.5 equiv) and **15** (1 equiv) provided intermediate **19**, which was oxidized in situ to give **20** in modest yield (Scheme 6). We realized that a major side product **21** was produced along with **19** in the Grignard reaction (~50:50, LC-MS), and although **21** could not be isolated in reasonable purity, the oxidized product **22** was isolated and structurally elucidated (see Supporting Information). This side reaction could be a result of a competing E2 elimination initiated by a reaction of the arylmagnesium species with the Cα hydrogen atom followed by elimination of the phenylmethanolate function as a leaving group (Scheme 7).



Scheme 7 Suggested mechanism for the side reaction observed in the Grignard reaction of **18**, resulting in **21** and subsequently **22** after DMP oxidation

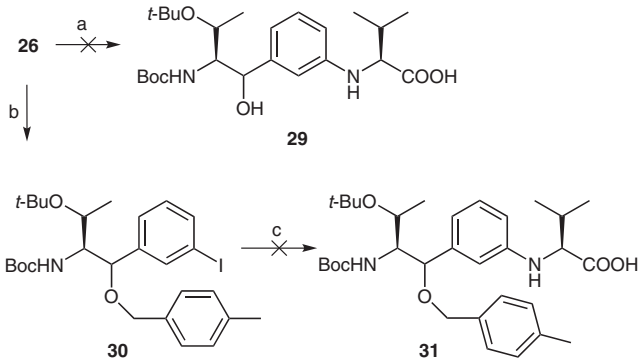
We therefore rationalized that replacing the *O*-benzyl protecting group with an *O-tert*-butyl group should not lead to the same degree of elimination, since 2-methylpropan-2-olate would be a much poorer leaving group than phenylmethanolate. Thus, *O-tert*-butyl-protected L-threoninal **25** was prepared in two steps from **23**, and the subsequent Grignard reaction with **25** and **15** provided **26**, which was oxidized to give **27**. The overall yield of **27** from **24** was increased from 42% to 66% by using both **25** and **26** without purification. Finally, **27** was aminated with L-valine using the mild (90 °C) version of the Ullmann–Goldberg reaction[11] to provide **28**, which upon treatment with trifluoroacetic acid gave target compound **1** (Scheme 8).



Scheme 8 *Reagents and conditions*: (a) (i) isobutyl chloroformate, NMM, THF, –20 °C, 30 min; (ii) $NaBH_4$, MeOH, –20 °C, 1 h, 93%; (b) DMP, 0.1% H_2O in CH_2Cl_2, r.t., 15 min, 75%; (c) 1,3-diiodobenzene (**15**), *i*-PrMgCl, THF, –78 °C → r.t., 45 min, 63%; (d) DMP, 0.1% H_2O in CH_2Cl_2, r.t., 15 min, 88%; (e) L-valine, CuI, K_2CO_3, DMF, 90 °C, 7 h, 40% (dr 78%); (f) TFA, r.t., 1 h, major epimer: 75% (dr 98%), minor epimer: 5% (dr 95%).

A general concern of our synthetic approach was the preservation of the stereochemistry of the two amino acid moieties. Up to compound **27** epimerization was prevented by avoiding flash chromatography of **25** and keeping the aldehyde intermediate cold during extraction and concentration;[19] however, LC-MS and analytical HPLC analysis revealed that **28** was obtained as a diastereomeric mixture of two species. By monitoring the reaction for the formation of **28** by LC-MS, we observed a slow but continuing epimerization of the precursor **27** during the reaction period, probably due to the basic conditions and elevated temperature. The reaction time for the synthesis of **28** was therefore settled at 7 hours as a compromise between optimizing the yield and avoiding excessive epimerization, which resulted in a 40% yield and a diastereomeric ratio of 78%. Although the use of DMF resulted in an increased yield of 40%, relative to 14% when DMA

was used, and microwave irradiation could reduce reaction time to 50 minutes (90 °C, 36% yield, dr 75%), this did not reduce the degree of epimerization. Instead, the epimeric mix of **28** was reacted with trifluoroacetic acid, and the epimers of **1** were isolated (dr 95–98%) by standard HPLC methods. Finally, in a principally different attempt to minimize epimerization, amination of alcohol **26** as well as the protected alcohol **30** was explored, assuming that these would be less susceptible to epimerization; however, none of the desired products, **29** and **31**, respectively, was obtained (Scheme 9), indicating the necessity for a carbonyl group *meta* to the halogen in order for the target compound to be obtained.

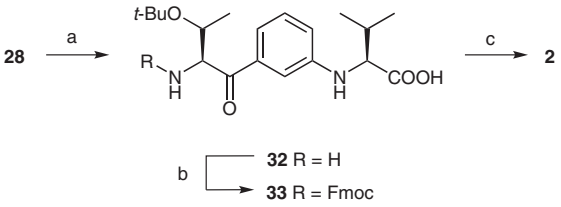


Scheme 9 *Reagents and conditions*: (a) and (c) L-valine, CuI, K_2CO_3, DMF, 90 °C, 24 h; (b) *p*-methylbenzyl bromide, 50% aq NaOH, toluene, r.t., 24 h, 81%.

In order to investigate if the tripeptide analogue **1** could be extended to longer peptide analogues using Fmoc-based solid-phase peptide chemistry, we selectively deprotected the *N-tert*-butoxycarbonyl group on precursor **28** (Scheme 10), without affecting the *O-tert*-butyl group, by treatment with HCl in isopropyl alcohol, which we found was much more selective than HCl in dioxane.[20] Subsequently, the primary amine was readily Fmoc-protected and **33** was loaded onto 2-chlorotrityl chloride resin. Standard Fmoc-based solid-phase peptide synthesis using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent, followed by cleavage from the resin and concomitant deprotection, and purification by HPLC provided compound **2** (Scheme 10).

Since peptide-based ligands generally are subject to enzymatic cleavage by proteases *in vivo*, which is often the major limiting factor for advanced biological studies,[21] we evaluated the *in vitro* blood plasma stability of **1**, **2**, TAV and IETAV using our previously described protocol.[22] We found that **1** degraded significantly slower than tripeptide TAV, with a 4.3-fold increase in half-life ($t_{1/2}$), and a similar effect was seen for **2** compared to pentapeptide IETAV (Table 1). Thus, clearly, the plasma stabilities of **1** and **2** have been significantly improved by the peptidomimetic modification.

Interestingly, we found that TAV was readily degraded at the N-terminal to the dipeptide AV, presumably by enzy-



Scheme 10 *Reagents and conditions*: (a) HCl, *i*-PrOH, r.t., 12 h, 78% (dr 75%); (b) Fmoc-OSu, 10% aq Na_2CO_3, dioxane, MeCN, r.t., 2 h (>90% conversion); (c) (i) 2-chlorotrityl chloride resin, DIPEA, CH_2Cl_2, DMF, r.t., 2 h; (ii) 20% piperidine in DMF, 2 × 10 min; (iii) Fmoc-Glu(*t*-Bu)-OH, HATU, collidine, r.t., 30 min; (iv) repeat (ii); (v) Fmoc-Ile-OH, HATU, collidine, r.t., 30 min; (vi) repeat (ii); (vii) TFA, r.t., 2 h, major epimer: 39% (dr 99%), epimeric mix: 9.9% (dr ~50%).

Table 1 Stability in Human Blood Plasma

Compound	$t_{1/2}$[a] (min)
TAV	30 ± 1.8
IETAV	47 ± 3.0
1	130 ± 5.5
2	80 ± 1.0

[a] Half-lives ($t_{1/2}$) are indicated as averages ± SEM, based on three independent measurements.

matic cleavage of the amide bond, and since a similar cleavage is not possible for **1**, this rationalizes the increased stability of **1**. The degradation of compound **1** was by a decarboxylation of the C-terminal, as suggested by LC-MS, and no further degradation was observed (up to 24 h). Compound **2** was somewhat less stable than **1**, resulting from a N-terminal cleavage of the isoleucine moiety, again indicating that the N-terminal part is the most sensitive for proteolytic degradation. The remaining tetrapeptide analogue was further decarboxylated, while no other degradation was observed over 24 hours.

In summary, we have established a practical route for obtaining benzene-based peptidomimetic scaffolds starting from 1,3-diiodobenzene, using a Grignard reaction and a copper-mediated Ullmann–Goldberg-type amination, respectively, for the introduction of the two amino acids into the aromatic ring system. Selective deprotection allowed further elaboration of **1** to the pentapeptide analogue **2** using Fmoc-based solid-phase peptide synthesis. Compared to their peptide congeners, both **1** and **2** possess increased proteolytic stability in human blood plasma. Thus, the synthesis presented herein could contribute to the development of future β-strand mimetics.

All reagents were obtained from commercial suppliers and used without further purification. THF was distilled from sodium/benzophenone and organometallic reagents were titrated prior to use. Melting points were determined on an MPA100 OptiMelt melting point apparatus and are presented as averaged values based on at least two measurements. ^{1}H NMR spectra were recorded on a Varian Mercury Plus (300 MHz) spectrometer and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 spectrometer (operating at

75 MHz), with tetramethylsilane as internal standard. Chemical shifts (δ) are reported as parts per million (ppm) and coupling constants (*J*) are reported in hertz (Hz). Elemental analyses were performed at the Department of Physical Chemistry, University of Vienna, Austria. High-resolution mass spectra (HRMS) were obtained using a Micromass Q-TOF 2 instrument and are all within 5 ppm of the theoretical values. LC-MS were obtained with an Agilent 6410 Triple Quadrupole mass spectrometer using electrospray ionization (ESI), coupled to an Agilent 1200 HPLC system (ESI-HPLC-MS) with a C18 reverse-phase column (Zorbax Eclipse XBD-C18, 4.6 × 50 mm), evaporative light-scattering detector (ELSD, Sedere Sedex 85) and a diode array detector (UV), using a linear gradient of the solvent system H_2O–MeCN–formic acid (A: 95:5:0.1; B: 5:95:0.086) with a flow rate of 1 mL/min. Preparative HPLC was performed on an Agilent 1100 system using a C18 reverse-phase column (Zorbax 300SB-C18, 21.2 × 250 mm) with a linear gradient of the solvent system H_2O–MeCN–TFA (A: 95:5:0.1; B: 5:95:0.1) with a flow rate of 20 mL/min. Analytical HPLC was performed on an Agilent 1100 system with a C18 reverse-phase column (Zorbax 300SB-C18, 4.6 × 150 mm) with a linear gradient of the above-mentioned solvent system of H_2O–MeCN–TFA with a flow rate of 1 mL/min.

_N_-(3-Iodophenyl)-2-nitrobenzenesulfonamide (4)

o-Nitrobenzenesulfonyl chloride (1.11 g, 5 mmol) was added in 10 portions over a period of 30 min under vigorous stirring to a soln of **3** (1.37 g, 6.25 mmol) and NaOAc (554 mg, 6.75 mmol) in EtOH–H_2O (1:1, 50 mL). The mixture was heated to 80 °C over a period of 30 min, then cooled, diluted with H_2O (50 mL) and acidified with 4 M aq HCl to pH ~2. The precipitate was collected by filtration and crystallized from EtOH (4 °C, 16 h) to give **4** (1.24 g, 60%) as yellowish crystals.

Mp 132.5–132.9 °C.

^{1}H NMR ($CDCl_3$): δ = 6.99 (t, *J* = 7.9 Hz, 1 H), 7.19 (ddd, *J* = 8.2, 2.2, 0.9 Hz, 1 H), 7.23 (br s, 1 H), 7.49 (ddd, *J* = 8.0, 1.6, 0.9 Hz, 1 H), 7.54 (t, *J* = 1.8 Hz, 1 H), 7.62 (td, *J* = 7.6, 1.5 Hz, 1 H), 7.71 (td, *J* = 7.7, 1.5 Hz, 1 H), 7.85 (dd, *J* = 2.8, 1.5 Hz, 1 H), 7.87 (dd, *J* = 2.8, 1.5 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 95.7, 119.8, 125.4, 128.9, 130.5, 131.6, 131.9, 133.4, 133.8, 135.6, 138.7, 148.5.

MS (EI): *m*/*z* (%) = 404 (42) [M$^+$], 218 (22), 186 (44), 91 (100), 64 (44).

Anal. Calcd for $C_{12}H_9IN_2O_4S$: C, 35.66; H, 2.24; N, 6.93; S, 7.93. Found: C, 35.72; H, 2.22; N, 6.75; S, 7.64.

_N_-Butyl-_N_-(3-iodophenyl)-2-nitrobenzenesulfonamide (5)

A flame-dried 2-neck round-bottom flask with a cooling condenser was charged with sulfonamide **4** (312 mg, 0.772 mmol) and Ph_3P (405 mg, 1.54 mmol), evacuated and a N_2 balloon was attached. Toluene (10 mL) and BuOH (140 μL, 1.54 mmol) were injected into the reaction mixture, which was then stirred and heated to 80 °C. Diisopropyl azodicarboxylate (DIAD; 300 μL, 1.54 mmol) was added slowly and the mixture was stirred for 2 h. The mixture was concentrated under reduced pressure and purified by flash chromatography (linear gradient EtOAc–heptane) to give **5** (153 mg, 43%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ = 0.83 (t, *J* = 7.2 Hz, 3 H), 1.20–1.40 (m, 4 H), 3.67 (t, *J* = 6.9 Hz, 2 H), 6.98 (t, *J* = 8.0 Hz, 1 H), 7.13 (ddd, *J* = 8.0, 2.0, 1.2 Hz, 1 H), 7.41–7.45 (m, 3 H), 7.50–7.61 (m, 3 H).

^{13}C NMR ($CDCl_3$): δ = 14.1, 19.9, 31.0, 52.3, 94.2, 124.3, 129.3, 131.1, 131.5, 131.9, 132.2, 134.2, 137.9, 138.5, 139.6, 148.4.

MS (EI): *m*/*z* (%) = 460 (6.3) [M$^+$], 417 (10), 232 (17), 186 (100), 105 (10), 91 (15), 77 (17), 64 (8.3), 51 (15).

Anal. Calcd for $C_{16}H_{17}IN_2O_4S$: C, 41.75; H, 3.72; N, 6.09; S, 6.97. Found: C, 41.76; H, 3.76; N, 6.04; S, 6.73.

_N_-(3-Iodophenyl)-_N_-isopropyl-2-nitrobenzenesulfonamide (6)

The procedure was the same as described for compound **5**, except that *i*-PrOH (118 μL, 1.54 mmol) was used instead of BuOH. The reaction mixture was concentrated under reduced pressure and purified by flash chromatography (linear gradient EtOAc–heptane) to give **6** (81.4 mg, 24%) as a white fluffy solid.

Mp 124.7–125.6 °C.

^{1}H NMR ($CDCl_3$): δ = 1.16 (d, *J* = 6.7 Hz, 6 H), 4.67 (septet, *J* = 6.5 Hz, 1 H), 7.06–7.10 (m, 2 H), 7.42–7.44 (m, 1 H), 7.50–7.61 (m, 2 H), 7.66 (t, *J* = 2.4 Hz, 1 H), 7.67–7.74 (m, 2 H).

^{13}C NMR ($CDCl_3$): δ = 22.7 (2 C), 52.6, 93.6, 124.3, 130.5, 131.5, 132.0, 132.6, 133.2, 133.9, 135.1, 138.4, 141.8, 147.9.

MS (EI): *m*/*z* (%) = 446 (16) [M$^+$], 431 (69), 404 (20), 245 (51), 186 (100), 133 (40), 91 (69), 76 (52), 64 (33), 51 (29).

Anal. Calcd for $C_{15}H_{15}IN_2O_4S$: C, 40.37; H, 3.39; N, 6.28; S, 7.19. Found: C, 40.43; H, 3.40; N, 6.16; S, 7.06.

(3-Fluorophenyl)(phenyl)methanol (8)

1-Fluoro-3-iodobenzene (**7**; 352 μL, 3 mmol) and THF (2 mL) were added to a flame-dried round-bottom flask under N_2 and the mixture was cooled to 0 °C. 1.24 M *i*-PrMgCl in THF (2.5 mL, 3.1 mmol) was added dropwise and the mixture was allowed to react for 30 min. PhCHO (273 μL, 2.7 mmol) was added and the mixture was stirred for 45 min, whilst allowing the temperature to increase to r.t. The reaction mixture was quenched with equal portions (5 mL) of sat. aq NH_4Cl, sat. aq $NaHCO_3$ and brine, and then extracted twice with EtOAc (2 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography (linear gradient EtOAc–heptane) to provide **8** (524 mg, 86%) as a colorless oil; purity >98% (GCMS, TLC).

^{1}H NMR ($CDCl_3$): δ = 2.48 (br s, 1 H), 5.78 (s, 1 H), 6.96 (t, *J* = 8.4 Hz, 1 H), 7.10–7.15 (m, 2 H), 7.25–7.36 (m, 6 H).[23]

MS (EI): *m*/*z* (%) = 202 (25) [M$^+$], 183 (10), 123 (42), 105 (100), 95 (15), 77 (40), 51 (19).

(3-Fluorophenyl)(phenyl)methanone (9)

H_2O (46 μL, 2.54 mmol) was thoroughly mixed with anhyd CH_2Cl_2 (45 mL), then slowly added dropwise (about 15 min) to a vigorously stirred soln of alcohol **8** (463.5 mg, 2.29 mmol), CH_2Cl_2 (1 mL) and 0.3 M DMP in CH_2Cl_2 (8.4 mL, 2.52 mmol). During the addition the solution turned white; the reaction was quenched by careful addition of sodium bisulfite (6 g, 58 mmol) in sat. aq $NaHCO_3$ (10 mL). The organic phase was washed with sat. aq $NaHCO_3$ (50 mL) and H_2O (50 mL), dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by flash chromatography (linear gradient EtOAc–heptane) to provide **9** (405 mg, 88%) as white crystals; purity >98% (GCMS, TLC).

Mp 51.8–52.2 °C (Lit.[24] 54.5–55 °C).

^{1}H NMR ($CDCl_3$): δ = 7.29 (tdd, *J* = 8.2, 2.7, 1.1 Hz, 1 H), 7.42–7.48 (m, 1 H), 7.48–7.53 (m, 3 H), 7.57 (dt, *J* = 7.6, 1.3 Hz, 1 H), 7.62 (dt, *J* = 7.3, 1.8 Hz, 1 H), 7.78–7.82 (m, 2 H).

MS (EI): *m*/*z* (%) = 200 (45) [M$^+$], 123 (28), 105 (100), 95 (26), 77 (51), 51 (23).

1-[3-(Butylamino)phenyl]ethanone (12)

A flame-dried 2-neck round-bottom flask with a cooling condenser was charged with CsOAc (877 mg, 4.56 mmol), CuI (347 mg, 1.82 mmol) and benzene (3.6 mL). The flask was evacuated and backfilled with N_2. DMF (6 mL), 3′-iodoacetophenone (125 μL, 0.912

mmol) and $BuNH_2$ (356 μL, 3.65 mmol) were added by syringe and the reaction mixture was stirred and heated to 90 °C for 5 h. The reaction mixture was cooled to r.t., quenched with sat. aq NH_4Cl–brine–H_2O (10:50:40, 100 mL) and extracted with EtOAc (50 mL). The organic phase was dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by flash chromatography (linear gradient EtOAc–heptane) to give **12** (112 mg, 64%) as a yellow solid.

Mp 29.8–31.4 °C.

^{1}H NMR ($CDCl_3$): δ = 0.97 (t, *J* = 7.2 Hz, 3 H), 1.39–1.51 (m, 2 H), 1.58–1.68 (m, 2 H), 2.58 (s, 3 H), 3.16 (t, *J* = 7.0 Hz, 2 H), 6.77–6.82 (m, 1 H), 7.17–7.27 (m, 3 H).

^{13}C NMR ($CDCl_3$): δ = 14.3, 20.7, 27.1, 31.8, 44.0, 111.6, 117.8 (2 C), 129.5, 138.3, 148.7, 198.8.

MS (EI): *m*/*z* (%) = 191 (36) [M$^+$], 148 (100), 106 (11), 91 (8.9), 77 (6.7), 65 (6.7), 32 (5.6).

Anal. Calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32. Found: C, 75.11; H, 8.57; N, 7.20.

(*S*)-2-[(3-Acetylphenyl)amino]-3-methylbutanoic Acid (14)

A flame-dried 2-neck round-bottom flask with a cooling condenser was charged with 3′-iodoacetophenone (787 mg, 3.2 mmol), L-valine (374 mg, 3.2 mmol), CuI (61 mg, 0.32 mmol) and K_2CO_3 (663 mg, 4.8 mmol). Anhyd DMF (4 mL) was added under N_2 atmosphere by syringe and the reaction was heated to 90 °C for 24 h. The reaction mixture was cooled to r.t. and quenched with H_2O (100 mL), which was followed by the addition of 4 M aq HCl to pH ~2 and dilution with EtOAc (50 mL). The aqueous phase was washed with EtOAc (50 mL) and the organic phases were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography [linear gradient EtOAc (10% AcOH)–heptane] to provide **14** (354 mg, 47%) as an orange oil.

^{1}H NMR ($CDCl_3$): δ = 1.07 (d, *J* = 6.7 Hz, 6 H), 2.16–2.27 (m, 1 H), 2.56 (s, 3 H), 3.96 (d, *J* = 5.3 Hz, 1 H), 6.82 (ddd, *J* = 7.9, 2.4, 1.2 Hz, 1 H), 7.21–7.33 (m, 3 H).

^{13}C NMR ($CDCl_3$): δ = 18.7, 19.6, 27.1, 31.7, 62.4, 112.8, 118.4, 118.9, 129.7, 138.2, 147.6, 178.3, 199.0.

MS (ESI): *m*/*z* (%) = 335.2 (11), 236.1 (100) [M + H]$^+$.

Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.01; H, 7.14; N, 6.01.

***tert*-Butyl [(2*R*,3*R*)-3-(Benzyloxy)-1-hydroxybutan-2-yl]carbamate (17)**

Boc-Thr(Bn)-OH (**16**; 8.7 g, 28 mmol) was dissolved in THF (80 mL). *N*-Methylmorpholine (3.3 mL, 29 mmol) was added and the mixture was cooled to –20 °C, treated with isobutyl chloroformate (4.4 mL, 29 mmol) and stirred for 30 min. The white precipitate was removed by filtration and rinsed with THF (30 mL). $NaBH_4$ (3.2 g, 84 mmol) was added to the filtrate; MeOH (100 mL) was carefully added at –20 °C. After 1 h the reaction was quenched with sat. aq NH_4Cl (50 mL), which was followed by the addition of EtOAc (50 mL). The two phases were separated and the organic layer was washed with brine (100 mL) and H_2O (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography (silica gel 60 pretreated with 5% Et_3N in EtOAc–heptane, linear gradient EtOAc–heptane) to give alcohol **17** (7.66 g, 88%) as a colorless oil; purity >98% (ELSD, TLC).

^{1}H NMR (DMSO-d_6): δ = 1.04 (d, *J* = 6.2 Hz, 3 H), 1.34 (s, 9 H), 3.29–3.37 (m, 1 H), 3.40–3.50 (m, 2 H), 3.56–3.64 (m, 1 H), 4.37 (d, *J* = 12.0 Hz, 1 H), 4.50 (d, *J* = 11.7 Hz, 1 H), 6.29 (d, *J* = 8.2 Hz, 1 H), 7.20–7.25 (m, 2 H), 7.26–7.29 (m, 3 H).[25]

MS (ESI): *m*/*z* (%) = 318.2 (100) [M + Na]$^+$, 262.2 (22), 196.2 (81).

***tert*-Butyl [(2*S*,3*R*)-3-(Benzyloxy)-1-oxobutan-2-yl]carbamate (18)**

H_2O (100 μL, 5.55 mmol) was thoroughly mixed with anhyd CH_2Cl_2 (100 mL), then slowly added dropwise (about 15 min) to a vigorously stirred soln of alcohol **17** (1.45 g, 5 mmol), CH_2Cl_2 (2 mL) and 0.3 M DMP in CH_2Cl_2 (18.3 mL, 5.5 mmol). During the addition the solution turned white; the reaction was quenched by careful addition of sodium bisulfite (13 g, 125 mmol) in sat. aq $NaHCO_3$ (20 mL). The organic phase was washed with sat. aq $NaHCO_3$ (100 mL), brine (100 mL) and twice with H_2O (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure at 0 °C. The product was purified by flash chromatography (silica gel 60 pretreated with 5% Et_3N in EtOAc–heptane, linear gradient EtOAc–heptane) to give aldehyde **18** (1.36 g, 93%) as a yellowish oil; purity >98% (ELSD, TLC).

^{1}H NMR (DMSO-d_6): δ = 1.14 (d, *J* = 6.2 Hz, 3 H), 1.36 (s, 9 H), 4.01–4.08 (m, 1 H), 4.12–4.18 (m, 1 H), 4.36 (d, *J* = 11.7 Hz, 1 H), 4.49 (d, *J* = 12.0 Hz, 1 H), 6.96 (d, *J* = 8.2 Hz, 1 H), 7.19–7.31 (m, 5 H), 9.47 (s, 1 H).[26]

MS (ESI): *m*/*z* (%) = 334.2 (9.8), 238.1 (33), 194.1 (100) [M – Boc + 2 H]$^+$, 150.1 (42).

***tert*-Butyl [(2*S*,3*R*)-3-(Benzyloxy)-1-(3-iodophenyl)-1-oxobutan-2-yl]carbamate (20) and *tert*-Butyl [(*Z*)-1-(3-Iodophenyl)-1-oxobut-2-en-2-yl]carbamate (22)**

1,3-Diiodobenzene (**15**; 1.036 g, 3.14 mmol) and THF (1.5 mL) were added to a flame-dried round-bottom flask under N_2 and the mixture was cooled to –78 °C. 2 M *i*-PrMgCl in THF (1.65 mL, 3.3 mmol) was added dropwise and the mixture was allowed to react for 15 min. Aldehyde **18** (461 mg, 1.57 mmol) in THF (1 mL) was added and the mixture was allowed to react for 1.5 h while the temperature increased to r.t. The reaction mixture was quenched with sat. aq NH_4Cl (10 mL), sat. aq $NaHCO_3$ (10 mL) and brine (10 mL), and then extracted with EtOAc (30 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude **19/21** (ratio: 45:55, LC-MS).

MS (ESI): *m*/*z* (%) = 520.2 (24) [M + Na]$^+$, 398.1 (100) [M – Boc + 2 H]$^+$ for **19**.

MS (ESI): *m*/*z* (%) = 412.1 (23) [M + Na]$^+$, 316.0 (100), 238.2 (34), 189.1 (83) for **21**.

This **19/21** mix was solubilized in CH_2Cl_2 (2 mL) and 0.3 M DMP in CH_2Cl_2 (5.8 mL, 1.74 mmol). H_2O (32 μL, 1.8 mmol) was thoroughly mixed with anhyd CH_2Cl_2 (32 mL) in a separation funnel, then added dropwise (15 min) to the vigorously stirred mixture of **19/21**. During the addition the solution turned white; the reaction was quenched by careful addition of sodium bisulfite (15 g, 143 mmol) in sat. aq $NaHCO_3$ (30 mL). The organic phase was washed with sat. aq $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. **20** and **22** were separated by flash chromatography (silica gel 60 pretreated with 5% Et_3N in EtOAc–heptane, linear gradient EtOAc–heptane).

20 was isolated by HPLC (213 mg, 27%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ = 1.29 (d, *J* = 6.2 Hz, 3 H), 1.47 (s, 9 H), 3.97 (dq, *J* = 6.3, 2.6 Hz, 1 H), 4.23 (d, *J* = 11.7 Hz, 1 H), 4.48 (d, *J* = 11.7 Hz, 1 H), 5.17 (dd, *J* = 8.9, 2.5 Hz, 1 H), 5.63 (d, *J* = 5.6 Hz, 1 H), 7.06–7.27 (m, 6 H), 7.76–7.87 (m, 2 H), 8.17 (t, *J* = 1.8 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 16.6, 28.8 (3 C), 60.7, 71.1, 74.4, 80.5, 94.9, 127.9, 128.1 (3 C), 128.7 (2 C), 130.6, 137.6, 137.7, 137.8, 142.3, 156.5, 196.9.

MS (ESI): *m*/*z* (%) = 518.2 (20) [M + Na]$^+$, 396.1 (100) [M – Boc + 2 H]$^+$, 352.1 (26).

Anal. Calcd for $C_{22}H_{26}INO_4$: C, 53.34; H, 5.29; N, 2.83. Found: C, 53.02; H, 5.01; N, 2.84.

HPLC gave the analytically pure **22** (28 mg, 4.6%) as a white freeze-dried solid; purity >98% (ELSD, analytical HPLC).

Mp 124.5–127.4 °C.

^{1}H NMR ($CDCl_3$): δ = 1.42 [s, 9 H, $(CH_3)_3$], 1.89 (d, *J* = 7.0 Hz, 3 H, =C–CH_3), 6.10 (q, *J* = 6.7 Hz, 1 H, =CH), 6.55 (br s, 1 H, NH), 7.16 (t, *J* = 7.8 Hz, 1 H, H-5), 7.69 (ddd, *J* = 7.8, 1.6, 1.0 Hz, 1 H, H-6), 7.84 (ddd, *J* = 7.9, 1.7, 1.0 Hz, 1 H, H-4), 8.05 (t, *J* = 1.6 Hz, 1 H, H-2).

^{13}C NMR ($CDCl_3$): δ = 14.6 (s, =C–CH_3), 28.5 [s, 3 C, C(CH_3)$_3$], 81.6 [s, $C(CH_3)_3$], 94.2 (s, C-3), 128.8 (s, C-6), 130.3 (s, C-5), 133.2 (s, =C–CH_3), 135.3 (s, C=C–CH_3), 138.3 (s, C-2), 139.4 (s, C-1), 141.3 (s, C-4), 153.6 [s, C(=O)NH], 191.9 [s, C(=O)C=C].

MS (ESI): *m*/*z* (%) = 410 (5.4) [M + Na]$^+$, 332 (46), 288 (100) [M – Boc + 2 H]$^+$.

Anal. Calcd for $C_{15}H_{18}INO_3$: C, 46.53; H, 4.69; N, 3.62. Found: C, 46.19; H, 4.30; N, 3.56.

tert-Butyl [(2*R*,3*R*)-3-*tert*-Butoxy-1-hydroxybutan-2-yl]carbamate (24)

Boc-Thr(*t*-Bu)-OH (**23**; 5 g, 18.2 mmol) was dissolved in THF (50 mL). *N*-Methylmorpholine (2.1 mL, 18.5 mmol) was added and the mixture was cooled to –20 °C, treated with isobutyl chloroformate (2.8 mL, 18.5 mmol) and stirred for 30 min. The white precipitate was removed by filtration and rinsed with THF (30 mL). $NaBH_4$ (2.06 g, 54 mmol) was added to the filtrate; MeOH (130 mL) was carefully added to the open flask at –20 °C. After 1 h the reaction was quenched with sat. aq NH_4Cl (50 mL), which was followed by the addition of EtOAc (50 mL). The two phases were separated and the organic phase was washed with brine (50 mL) and H_2O (100 mL). The combined aqueous phases were washed three times with EtOAc–heptane (1:1, 50 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography (silica gel 60 pretreated with 5% Et_3N in EtOAc–heptane; EtOAc–heptane, 1:1) to give alcohol **24** (4.42 g, 93%) as white flakes.

Mp 95.5–96.4 °C.

^{1}H NMR ($CDCl_3$): δ = 1.17 (d, *J* = 6.2 Hz, 3 H), 1.20 (s, 9 H), 1.45 (s, 9 H), 2.92 (br s, 1 H), 3.48–3.57 (m, 1 H), 3.62–3.67 (m, 2 H), 3.88–3.96 (m, 1 H), 5.01 (d, *J* = 7.3 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 20.5, 28.8, 29.1, 57.1, 64.1, 67.3, 74.5, 79.8, 156.9.

MS (ESI): *m*/*z* (%) = 545.4 (37) [2 M + Na]$^+$, 284.2 (54) [M + Na]$^+$, 150.1 (100).

Anal. Calcd for $C_{13}H_{27}NO_4$: C, 59.74; H, 10.41; N, 5.36. Found: C, 59.36; H, 10.18; N, 5.49.

tert-Butyl [(2*S*,3*R*)-3-*tert*-Butoxy-1-oxobutan-2-yl]carbamate (25)

H_2O (100 μL, 5.55 mmol) was thoroughly mixed with anhyd CH_2Cl_2 (100 mL), then slowly added dropwise (about 15 min) to a vigorously stirred soln of alcohol **24** (1.31 g, 5 mmol), CH_2Cl_2 (2 mL) and 0.3 M DMP in CH_2Cl_2 (18.3 mL, 5.5 mmol). During the addition the solution turned white; the reaction was quenched by careful addition of sodium bisulfite (13 g, 125 mmol) in sat. aq $NaHCO_3$ (20 mL). The organic phase was washed with sat. aq $NaHCO_3$ (100 mL), brine (100 mL) and twice with H_2O (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure at 0 °C. The analytically pure product was obtained by flash chromatography (silica gel 60 pretreated with 5% Et_3N in heptane, heptane as eluent) to give **25** (972 mg, 75%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ = 1.14 (d, *J* = 6.2 Hz, 3 H), 1.20 (s, 9 H), 1.46 (s, 9 H), 4.18 (dd, *J* = 8.0, 3.5 Hz, 1 H), 4.24–4.31 (m, 1 H), 5.39 (d, *J* = 7.0 Hz, 1 H), 9.67 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ = 20.0, 28.7 (3 C), 28.8 (3 C), 65.5, 66.7, 74.9, 80.2, 156.2, 201.7.

MS (ESI): *m*/*z* (%) = 159 (29) [M – Boc + H]$^+$, 130 (27), 118 (18), 103 (78), 86 (9), 74 (62), 57 (100).

Anal. Calcd for $C_{13}H_{25}NO_4$: C, 60.21; H, 9.72; N, 5.40. Found: C, 60.21; H, 9.78; N, 5.49.

tert-Butyl [(2*R*,3*R*)-3-*tert*-Butoxy-1-hydroxy-1-(3-iodophenyl)butan-2-yl]carbamate (26)

1,3-Diiodobenzene (**15**; 1.98 g, 6 mmol) and THF (3 mL) was added to a flame-dried round-bottom flask under N_2 and the mixture was cooled to –78 °C. 2 M *i*-PrMgCl in THF (3.1 mL, 6.2 mmol) was added dropwise and the mixture was stirred for 15 min. Aldehyde **25** (648 mg, 2.5 mmol) in THF (4 mL) was added, which was followed by stirring of the mixture for 45 min while the temperature increased to r.t. The reaction mixture was quenched with equal portions (20 mL) of sat. aq NH_4Cl, sat. aq $NaHCO_3$ and brine, and then extracted with EtOAc (2 × 40 mL). The combined organic layers were washed with H_2O (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography (silica gel 60 pretreated with 5% Et_3N in EtOAc–heptane, linear gradient EtOAc–heptane) to give **26** (733 mg, 63%) as a sticky white solid; purity >98% (ELSD, TLC).

Mp 107.2–107.7 °C.

^{1}H NMR ($CDCl_3$): δ = 1.22 (d, *J* = 6.2 Hz, 3 H), 1.28 (s, 9 H), 1.34 (s, 9 H), 3.62 (dt, *J* = 10.0, 2.9 Hz, 1 H), 4.04–4.11 (m, 1 H), 4.32 (br s, 1 H), 4.87 (d, *J* = 2.4 Hz, 1 H), 5.20 (d, *J* = 9.7 Hz, 1 H), 7.02 (t, *J* = 7.8 Hz, 1 H), 7.26 (d, *J* = 7.0 Hz, 1 H), 7.55 (d, *J* = 7.9 Hz, 1 H), 7.73 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ = 20.8, 28.7 (3 C), 29.4 (3 C), 60.5, 71.4, 75.1, 75.3, 79.9, 94.7, 125.8, 130.2, 135.5, 136.7, 144.3, 156.9.

MS (ESI): *m*/*z* (%) = 949.4 (23) [2 M + Na]$^+$, 486.1 (15) [M + Na]$^+$, 334.0 (100), 290.0 (39).

tert-Butyl [(2*S*,3*R*)-3-*tert*-Butoxy-1-(3-iodophenyl)-1-oxobutan-2-yl]carbamate (27)

H_2O (27 μL, 1.5 mmol) was thoroughly mixed with anhyd CH_2Cl_2 (27 mL), then slowly added dropwise (about 15 min) to a vigorously stirred soln of alcohol **26** (633 mg, 1.37 mmol), CH_2Cl_2 (2 mL) and 0.3 M DMP in CH_2Cl_2 (5 mL, 1.5 mmol). During the addition the solution turned white; the reaction was quenched by careful addition of sodium bisulfite (3.5 g, 34 mmol) in sat. aq $NaHCO_3$ (10 mL). The organic phase was washed with sat. aq $NaHCO_3$ (30 mL) and twice with H_2O (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography (silica gel 60 pretreated with 5% Et_3N in EtOAc–heptane, linear gradient EtOAc–heptane) to give **27** (556 mg, 88%) as a thick colorless oil.

^{1}H NMR ($CDCl_3$): δ = 1.05 (s, 9 H), 1.19 (d, *J* = 6.0 Hz, 3 H), 1.45 (s, 9 H), 4.01–4.04 (m, 1 H), 5.08 (dd, *J* = 8.7, 3.0 Hz, 1 H), 5.59 (d, *J* = 8.4 Hz, 1 H), 7.20 (t, *J* = 7.5 Hz, 1 H), 7.87–7.91 (m, 2 H), 8.28 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ = 20.5, 28.7 (3 C), 28.8 (3 C), 61.3, 68.1, 74.7, 80.2, 94.6, 128.3, 130.6, 138.1, 138.3, 142.4, 156.2, 197.5.

MS (ESI): *m*/*z* (%) = 484.2 (49) [M + Na]$^+$, 306.0 (100) [M – Boc – *t*-Bu + 3 H]$^+$.

Anal. Calcd for $C_{19}H_{28}INO_4$: C, 49.47; H, 6.12; N, 3.04. Found: C, 49.16; H, 5.59; N, 2.85.

(*S*)-2-(3-[(2*S*,3*R*)-3-*tert*-Butoxy-2-(*tert*-butoxycarbonylamino)butanoyl]phenylamino)-3-methylbutanoic Acid (28)

A flame-dried round-bottom flask was quickly charged with **27** (677 mg, 1.47 mmol), L-valine (172 mg, 1.47 mmol), CuI (28 mg, 0.15 mmol), K_2CO_3 (308 mg, 2.2 mmol) and DMF (2 mL). The flask was fitted with a condenser, evacuated and backfilled with N_2. The reaction mixture was heated to 90 °C for 7 h, which was followed by cooling and quenching with H_2O (30 mL). EtOAc (30 mL) was added and the aqueous phase was acidified (pH ~2) and shaken with the organic phase. The aqueous phase was isolated and washed twice with EtOAc (30 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography [linear gradient EtOAc (10% AcOH)–heptane] to give **28** (266 mg, 40%, dr 78%) as a yellow solid; purity >98% (ELSD).

Mp 77.1–77.8 °C.

^{1}H NMR ($CDCl_3$): δ = 0.96 (s, 9 H), 1.05 (d, *J* = 6.5 Hz, 3 H), 1.08 (d, *J* = 6.7 Hz, 3 H), 1.23 (d, *J* = 6.2 Hz, 3 H), 1.46 (s, 9 H), 2.17–2.28 (m, 1 H), 3.99–4.05 (m, 2 H), 5.21 (dd, *J* = 9.4, 2.1 Hz, 1 H), 5.72 (d, *J* = 9.4 Hz, 1 H), 6.81 (dt, *J* = 6.9, 2.1 Hz, 1 H), 7.21–7.28 (m, 2 H), 7.37 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ = 18.7, 19.3, 21.3, 28.68 (3 C), 28.73 (3 C), 31.7, 61.2, 62.2, 68.2, 74.5, 80.8, 114.5, 118.3, 119.0, 130.1, 136.7, 147.7, 157.0, 176.9, 198.5.

MS (ESI): *m*/*z* (%) = 339.1 (26), 295.1 (100) [M – Boc – *t*-Bu + 3H]$^+$, 251.1 (15).

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{24}H_{39}N_2O_6$: 451.2808; found: 451.2796.

(*S*)-2-(3-[(2*S*,3*R*)-2-Amino-3-hydroxybutanoyl]phenylamino)-3-methylbutanoic Acid (1)

A mixture of TFA–triisopropylsilane–H_2O (90:5:5, 2 mL) and **28** (105 mg, 0.233 mmol) was stirred at r.t. for 1 h, then concentrated, followed by three cycles of addition/evaporation of Et_2O (10 mL). HPLC gave trifluoroacetate salts of compound **1** (major epimer: 71 mg, 75%, dr 98%; minor epimer: 5.0 mg, 5%, dr 95%) as yellow freeze-dried solids.

Data for the major epimer: purity >98% (ELSD).

Mp 56.9–58.0 °C.

^{1}H NMR (D_2O): δ = 0.89 (d, *J* = 5.5 Hz, 3 H), 0.92 (d, *J* = 5.5 Hz, 3 H), 1.19 (d, *J* = 6.5 Hz, 3 H), 2.00–2.12 (m, 1 H), 3.79 (d, *J* = 5.9 Hz, 1 H), 4.22–4.29 (m, 1 H), 4.91 (d, *J* = 2.6 Hz, 1 H), 6.96–7.01 (m, 1 H), 7.12 (s, 1 H), 7.24–7.30 (m, 2 H).

^{13}C NMR (D_2O): δ = 18.2, 18.6, 19.6, 30.8, 61.2, 63.9, 66.0, 113.6, 120.5, 121.5, 130.6, 134.5, 147.3, 177.5, 196.5.

MS (ESI): *m*/*z* (%) = 295.1 (100) [M + H]$^+$, 251.1 (9.6).

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{23}N_2O_4$: 295.1658; found: 295.1674.

***tert*-Butyl [(2*S*,3*R*)-3-*tert*-Butoxy-1-(3-iodophenyl)-1-(4-methylbenzyloxy)butan-2-yl]carbamate (30)**

Toluene (20 mL) and 50% aq NaOH (15 mL) were added to alcohol **26** (576 mg, 1.24 mmol). The solution was vigorously stirred and treated with *p*-methylbenzyl bromide (247 mg, 1.34 mmol) and tetrabutylammonium hydrogen sulfate (48 mg, 0.14 mmol) for 24 h, which was followed by the addition of H_2O (30 mL) and extraction with Et_2O (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by flash chromatography (silica gel 60 pretreated with 5% Et_3N in EtOAc–heptane, linear gradient EtOAc–heptane) to give **30** (566 mg, 81%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ = 1.16 (d, *J* = 6.5 Hz, 3 H), 1.20 (s, 9 H), 1.38 (s, 9 H), 2.35 (s, 3 H), 3.58–3.73 (m, 1 H), 4.25–4.34 (m, 1 H), 4.66 (d, *J* = 2.6 Hz, 1 H), 5.12 (d, *J* = 9.4 Hz, 2 H), 7.06 (t, *J* = 7.6 Hz, 1 H), 7.13–7.19 (m, 5 H), 7.27 (d, *J* = 7.6 Hz, 1 H), 7.59 (d, *J* = 7.6 Hz, 1 H), 7.68 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ = 19.3, 21.5, 28.8 (3 C), 29.1 (3 C), 60.7, 67.7, 71.3, 74.2, 78.2, 79.3, 94.7, 126.5, 128.4 (2 C), 129.4 (2 C), 130.5, 135.2, 136.3, 137.0, 137.8, 143.9, 156.0.

MS (ESI): *m*/*z* (%) = 590.2 (28) [M + Na]$^+$, 468.1 (22) [M – Boc + 2 H]$^+$, 412.1 (100) [M – Boc – *t*-Bu + 3 H]$^+$.

Anal. Calcd for $C_{27}H_{38}INO_4$: C, 57.14; H, 6.75; N, 2.47. Found: C, 57.20; H, 6.58; N, 2.28.

(*S*)-2-(3-[(2*S*,3*R*)-2-Amino-3-*tert*-butoxybutanoyl]phenylamino)-3-methylbutanoic Acid (32)

A mixture of 1.25 M HCl in *i*-PrOH (100 mL, 125 mmol) and **28** (320 mg, 0.71 mmol) was stirred at r.t. for 12 h, then concentrated under reduced pressure and coevaporated twice with CH_2Cl_2 (30 mL). Compound **32** was obtained as the trifluoroacetate salt by HPLC purification (257 mg, 78%, dr 75%) as a yellow freeze-dried solid; purity >98% (ELSD).

Mp 85.5–86.7 °C.

^{1}H NMR ($CDCl_3$): δ = 0.93 (s, 9 H), 1.01 (d, *J* = 6.7 Hz, 3 H), 1.04 (d, *J* = 6.7 Hz, 3 H), 1.19 (s, 2 H), 1.35 (d, *J* = 6.5 Hz, 3 H), 2.08–2.19 (m, 1 H), 3.81 (d, *J* = 5.6 Hz, 1 H), 4.10–4.18 (m, 1 H), 4.91 (br s, 1 H), 5.02 (d, *J* = 4.1 Hz, 1 H), 6.80–6.89 (m, 1 H), 7.04–7.19 (m, 3 H).

^{13}C NMR (CD_3OD): δ = 18.2, 18.7, 20.5, 27.6 (3 C), 31.2, 61.3, 62.7, 66.4, 74.9, 112.2, 117.6, 119.4, 129.8, 134.7, 149.3, 175.8, 194.3.

MS (ESI): *m*/*z* (%) = 351.2 (100) [M + H]$^+$, 295.1 (27) [M – *t*-Bu + 2 H]$^+$.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{19}H_{31}N_2O_4$: 351.2284; found: 351.2299.

(*S*)-4-[(2*S*,3*S*)-2-Amino-3-methylpentanamido]-5-([(2*S*,3*R*)-1-[3-([(*S*)-1-carboxy-2-methylpropyl]amino)phenyl]-3-hydroxy-1-oxobutan-2-yl]amino)-5-oxopentanoic Acid (2)

Compound **32** (451 mg, 0.97 mmol) was dissolved as the trifluoroacetate salt in dioxane–MeCN (1:1, 5 mL), then 10% aq Na_2CO_3 (15 mL) was added under vigorous stirring. Fmoc-OSu (492 mg, 1.46 mmol) in dioxane (3 mL) was added slowly. The orange slurry mixture was stirred for 2 h. H_2O (60 mL) was added and the aqueous layer was extracted twice with EtOAc (60 mL). The aqueous phase was acidified to pH ~2 with 4 M aq HCl and extracted once with EtOAc (60 mL). The combined organic layers were concentrated under reduced pressure, dissolved in H_2O–MeCN (1:1, 60 mL) and freeze-dried to give crude **33** as a yellow solid; purity ~90% (ELSD).

MS (ESI): *m*/*z* (%) = 595.2 (12) [M + Na]$^+$, 517.2 (100) [M – *t*-Bu + 2 H]$^+$, 473.2 (17).

2-Chlorotrityl chloride resin (142 mg, 0.22 mmol) was swelled in anhyd CH_2Cl_2 (2 mL) for 15 min, which was followed by draining and incubation with half the portion of crude **33** (~0.44 mmol) in a mixture of CH_2Cl_2–DMF (2:1, 2 mL) and DIPEA (145 μL, 0.87 mmol) for 2 h. The resin was capped with MeOH (CH_2Cl_2–MeOH–DIPEA, 17:2:1) three times for 2 min. Fmoc deprotection was performed with 20% piperidine in DMF (2 × 10 min), and coupling of the consecutive amino acid was carried out with HATU and collidine (resin–amino acid–HATU–collidine 1:4:4:6) for 30 min. The resin was cleaved with 5% H_2O and 5% triisopropylsilane in TFA for 2 h, which was followed by concentration under reduced pressure and coevaporation with CH_2Cl_2 (2 × 20 mL) to obtain crude **2** (dr 85%, ELSD). HPLC of half the portion of crude **2** gave the freeze-dried trifluoroacetate salt of compound **2** as the major epimer

(28 mg, 39%, dr 99%) and as the epimeric mix (7.1 mg, 9.9%, dr ~50%) as yellowish solids.

Data for major epimer: purity >98% (ELSD).

Mp 111.0–114.5 °C.

^{1}H NMR (D_2O): δ = 0.78 (t, *J* = 7.3 Hz, 3 H), 0.85 (d, *J* = 6.7 Hz, 3 H), 0.91 (d, *J* = 4.9 Hz, 3 H), 0.93 (d, *J* = 4.8 Hz, 3 H), 1.07 (d, *J* = 6.2 Hz, 3 H), 1.28–1.42 (m, 2 H), 1.76–1.92 (m, 3 H), 2.01–2.11 (m, 1 H), 2.17–2.27 (m, 2 H), 3.75 (d, *J* = 5.3 Hz, 1 H), 3.77 (d, *J* = 5.9 Hz, 1 H), 4.21–4.28 (m, 1 H), 4.40 (t, *J* = 7.3 Hz, 1 H), 5.21 (d, *J* = 2.6 Hz, 1 H), 6.91–6.97 (m, 1 H), 7.08 (br s, 1 H), 7.23–7.28 (m, 2 H).

MS (ESI): *m*/*z* (%) = 1073.5 (51) $[2\,M + H]^+$, 537.2 (100) $[M + H]^+$, 269.1 (6) $[M + 2\,H]^{2+}$.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{26}H_{41}N_4O_8$: 537.2924; found: 537.2922.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis. Included are experimental details for the human blood plasma stability assay, for the alkylation experiments and for the preparation of the STABASE adduct, ^{1}H and ^{13}C NMR spectra of all purified compounds and 2D NMR spectra of compound **22**.

Acknowledgment

This work was supported by the Lundbeck Foundation (K.S.), the Drug Research Academy, Faculty of Pharmaceutical Sciences, University of Copenhagen, Denmark (A.B.), and the Danish Council for Independent Research, Technology and Production Sciences (A.B.). We thank Nicolai Stuhr-Hansen for a critical reading of the manuscript.

References

(1) (a) Loughlin, W. A.; Tyndall, J. D.; Glenn, M. P.; Hill, T. A.; Fairlie, D. P. *Chem. Rev.* **2004**, *104*, 6085. (b) Loughlin, W. A.; Tyndall, J. D.; Glenn, M. P.; Hill, T. A.; Fairlie, D. P. *Chem. Rev.* **2010**, *110*, PR32.
(2) Remaut, H.; Waksman, G. *Trends Biochem. Sci.* **2006**, *31*, 436.
(3) Giannis, A.; Kolter, T. *Angew. Chem. Int. Ed.* **1993**, *32*, 1244.
(4) (a) Hruby, V. J.; Balse, P. M. *Curr. Med. Chem.* **2000**, *7*, 945. (b) Hruby, V. J. *Nat. Rev. Drug Discovery* **2002**, *1*, 847.
(5) Bach, A.; Chi, C. N.; Olsen, T. B.; Pedersen, S. W.; Røder, M. U.; Pang, G. F.; Clausen, R. P.; Jemth, P.; Strømgaard, K. *J. Med. Chem.* **2008**, *51*, 6450.
(6) Ripka, A. S.; Rich, D. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 441.
(7) Blomberg, D.; Brickmann, K.; Kihlberg, J. *Tetrahedron* **2006**, *62*, 10937.
(8) Blomberg, D.; Fex, T.; Xue, Y.; Brickmann, K.; Kihlberg, J. *Org. Biomol. Chem.* **2007**, *5*, 2599.
(9) Saitton, S.; Kihlberg, J.; Luthman, K. *Tetrahedron* **2004**, *60*, 6113.
(10) Mitsunobu, O. *Synthesis* **1981**, 1.
(11) Ma, D.; Zhang, Y.; Yao, J.; Wu, S.; Tao, F. *J. Am. Chem. Soc.* **1998**, *120*, 12459.
(12) Okano, K.; Tokuyama, H.; Fukuyama, T. *Org. Lett.* **2003**, *5*, 4987.
(13) Sokolov, V.; Butkevich, A.; Yuskovets, V.; Tomashevskii, A.; Potekhin, A. *Russ. J. Org. Chem.* **2005**, *41*, 1023.
(14) Varchi, G.; Kofink, C.; Lindsay, D. M.; Ricci, A.; Knochel, P. *Chem. Commun.* **2003**, 396.
(15) (a) Kopp, F.; Krasovskiy, A.; Knochel, P. *Chem. Commun.* **2004**, 2288. (b) Kopp, F.; Wunderlich, S.; Knochel, P. *Chem. Commun.* **2007**, 2075. (c) Boymond, L.; Rottländer, M.; Cahiez, G.; Knochel, P. *Angew. Chem. Int. Ed.* **1998**, *37*, 1701.
(16) (a) Stoll, A. H.; Knochel, P. *Org. Lett.* **2008**, *10*, 113. (b) Djuric, S.; Venit, J.; Magnus, P. *Tetrahedron Lett.* **1981**, *22*, 1787.
(17) Wen, J. J.; Crews, C. M. *Tetrahedron: Asymmetry* **1998**, *9*, 1855.
(18) (a) Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, *59*, 7549. (b) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
(19) Lubell, W. D.; Rapoport, H. *J. Am. Chem. Soc.* **1987**, *109*, 236.
(20) Han, G.; Tamaki, M.; Hruby, V. J. *J. Pept. Res.* **2001**, *58*, 338.
(21) Harris, J. M.; Martin, N. E.; Modi, M. *Clin. Pharmacokinet.* **2001**, *40*, 539.
(22) Bach, A.; Chi, C. N.; Pang, G. F.; Olsen, L.; Kristensen, A. S.; Jemth, P.; Strømgaard, K. *Angew. Chem. Int. Ed.* **2009**, *48*, 9685.
(23) Nolte, C.; Mayr, H. *Eur. J. Org. Chem.* **2010**, 1435.
(24) (a) Garth, R.; Yuho, P.; Taft, T.; Taft, R. W. *J. Am. Chem. Soc.* **1967**, *89*, 2391. (b) Buu-Hoi, N. P.; Lescot, E. Jr.; Xuong, N. D. *J. Org. Chem.* **1957**, *22*, 1057.
(25) Nakamura, Y.; Hirai, M.; Tamotsu, K.; Yonezawa, Y.; Shin, C. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 13699.
(26) Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676.