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Chemical reactivity of 6-diazo-5-oxo-L-norleucine (DON) catalyzed by metalloporphyrins (Fe,Ru)

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ABSTRACT

The transfer of the metallocarbene derived from *N*- and *O*-protected 6-diazo-5-oxo-L-norleucine (DON) catalyzed by metalloporphyrins undergoes dimerization, cyclopropanation, N–H and S–H insertion reactions, respectively. An efficient and direct synthesis of 5-oxo-L-pipecolic acid from DON is described from unprotected 6-diazo-5-oxo-L-norleucine.

1. Introduction

6-Diazo-5-oxo-L-norleucine (commonly known as DON) is an antibiotic that was isolated from *Streptomyces* in 1956.[1] It is the diazo analog of L-glutamic acid and has been shown to interfere with a number of biological pathways, including purine, pyrimidine, and protein synthesis where glutamine is used as a nitrogen source.[2] DON acts as a suicide inhibitor of glutaminase/asparaginase[3] and has subsequently been used in a variety of applications including labeling of the active sites of enzymes[4] and possibly as anticancer drug.[2,5] Combination therapy of animal tumors with L-asparaginase and 6-diazo-5-oxo-L-norleucine, was also suggested, more than thirty years ago,[6] and is now starting a new development.[7]

For the objective of understanding better the in vivo reactivity, we have studied the chemical reactivity of protected DON with metalloporphyrins as models of heme enzymes. Actually, possible interaction of diazoketones with enzymes such as cytochrome P450 has been previously suggested.[8,9] It is also expected that metalloporphyrins (Fe,Ru) are well suited as catalysts for intermolecular carbene transfer using polyfunctionnalyzed diazo derivatives as reagents due to good selectivity. Here we report that metallocarbene transfers derived from *N*- and *O*-protected 6-diazo-5-oxo-L-norleucine (DON) catalyzed by metalloporphyrins are possible giving intermolecular reactions such as dimerization, cyclopropanation, N–H and S–H insertion reactions. We also report intramolecular N–H insertion with unprotected 6-diazo-5-oxo-L-norleucine, which led to 5-keto-L-pipecolic acid.

2. Results

The antibiotic 6-diazo-5oxo-L-norleucine has been synthesized several times from the *N*-protected acyl chloride and diazomethane, but in low overall yields.[10] Herein, we used a more recent method reported by Coutts and Saint,[11] in which the lithium salt of trimethylsilyldiazomethane was reacted with pyroglutamates. In our hands, the *N*- and *O*-protected diazo derivative was prepared in 50% yield (Scheme 1). Using a similar route, the unprotected diazo amino acid was also prepared (Scheme 2, 52% yield) (see also Experimental section).

To demonstrate the presence of possible metallocarbene intermediate, first cyclopropanation reaction was investigated (Scheme 3). The results are summarized in Table 1. Thus to evaluate the reactivity of *N*- and *O*-protected 6-diazo-5-oxo-L-norleucine compound, its ruthenium-catalyzed decomposition was first examined in the presence of styrene in toluene at room temperature in the presence of tetraphenylporphyrin ruthenium carbon monoxide **1** (Fig. 1) as catalyst (Table 1, entry 1). The cyclopropane was formed with 72% yield and high diastereoselectivity (trans/cis=99/1) with a concomitant formation of the dimer (21%), resulting from coupling of two carbene precursors. An increased yield (85%) with a selectivity (trans/cis=91/9) was observed using tetraphenylporphyrin iron chloride **3** instead of the ruthenium complex **1**, as catalyst. As previously reported by Woo and coll.[12,13] with ethyl

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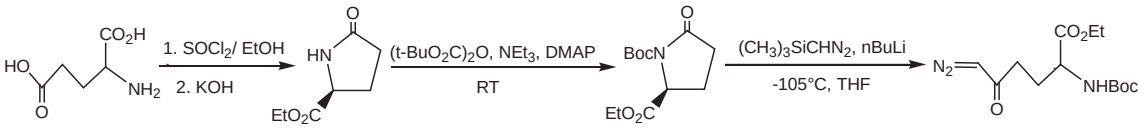
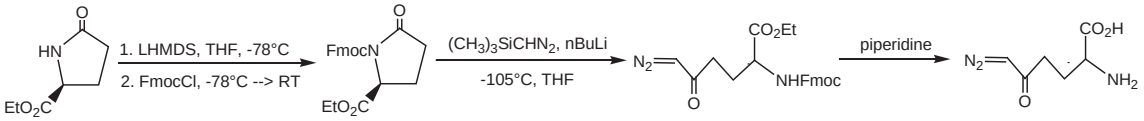


Scheme 1.



Scheme 2.

Scheme 3.

Table 1
Cyclopropanation of styrene with $N_2CHCO(CH_2)_2CH(CO_2Et)NHBoc$ catalyzed by Ru(CO)(TPP) (**1**), (−)-Ru(CO)(Halt) (**2**), Fe(Cl)(TPP) (**3**), (−)-Fe(Cl)(Halt) (**4**)[a]

Enty	Catalyst	Time (h)	$Yield_{trans+cis}$ (%)[b]	Ratio $_{trans/cis}$	ee $_{trans}$ (%)[c]	Dimer (%)
1	**1**	5	72	99/1	—	21
2	**2**[e]	5	75	99/1	80	18
3	**3**	2	85	91/9	—	7
4[d]	**4**	2	95	95/5	80	<5

[a] A molar ratio of 1:200:1000 for catalyst: diazo: styrene was employed at ambient temperature in toluene.
[b] Yields were determined by isolation of cyclopropanes by column chromatography on silica gel.
[c] ees were determined by chiral HPLC using a Chiralcel OD column.
[d] At 40 °C.
[e] The other enantiomer (+)-Ru(CO)(Halt) gave an ee=68%.

diazoacetate, we took benefit of the addition of cobaltocene to increase the reactivity. Actually, the absence of cobaltocene did not give any cyclopropanation reaction, probably due to the difficulty of reducing the ferric state to the ferrous state. As expected with **1** or **3**, no enantiomeric excess is observed.

We then investigated the cyclopropanation with chiral Halterman porphyrin ruthenium carbon monoxide **2** (Fig. 1) (Table 1). With styrene, the cyclopropane was formed with 75% yield, 99/1 trans/cis ratio and 80% enantioselectivity for the trans isomer (Table 1, entry 2). We also investigated the asymmetric cyclopropanation catalyzed by chiral Halterman porphyrin iron chloride **4** (Table 1). As shown in Table 1, the chemical yields are higher (~95%) with iron porphyrins than those obtained with ruthenium porphyrins **1** or **2** (72–75%) and the enantioselectivity was quite good, 80% ee. It should be noted that other chiral metalloporphyrins derived from

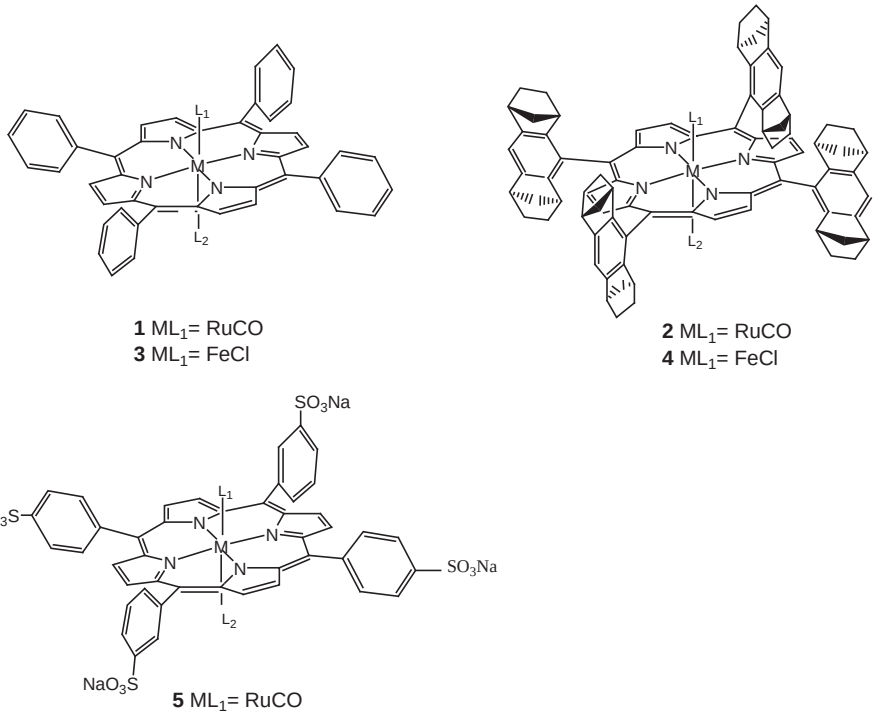


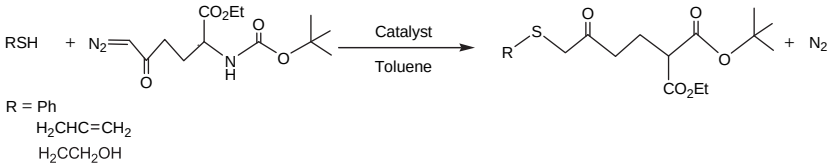
Figure 1. Catalysts used in the reactions of cyclopropanation, N–H and S–H insertions with $N_2CHCO(CH_2)_2CH(CO_2Et)NHBoc$.

aminophenylporphyrins[14] have been reported to give cyclopropanes with high enantiomeric excesses, when ethyl diazoacetate was used as reagent.[15,16]

Since peptidyl diazomethyl ketones appeared initially to be specific inactivators of cysteine proteinases,[17,18] insertion of DON into S–H bonds, catalyzed by metalloporphyrins, was assayed (Scheme 4). The different results obtained with **1** or **3** as catalysts are summarized in Table 2. Treatment of thiophenol with *N*-*tert*-butoxycarbonyl-6-diazo-5-oxo-L-norleucine methyl ester catalyzed by complex **1** and **3** gave insertion of the diazo derivative into the S–H bond with 85 and 51% yield, respectively. To complete the reactivity of this diazoketone, an investigation of the competition between the cyclopropanation of alkenes and the insertion reaction catalyzed by complex **1** or **3** was also undertaken (entries 3 and 4, Table 2). Only the S–H insertion compound is observed by ^{1}H NMR when allyl sulphide is the substrate with 87 and 58% yield, respectively. It should be noted that a preference for insertion has already been observed in ruthenium-catalyzed reaction of diazoesters with unsaturated thiols.[19]

DON and its next smaller homolog 5-diazo-4-oxo-L-norvaline, act as suicide inhibitors of glutaminase/asparaginases and were proposed to form an α-keto ether linkage through the reaction with the OH group of a threonine in the active site.[3] Thus to fully characterize the catalytic property of the ruthenium and iron porphyrin complexes, an investigation of the competition between the S–H and O–H insertions was also undertaken to see if O–H insertion was also possible. As can be seen in Table 2 (entries 5 and 6), we observed only S–H insertion with 2-mercaptoethanol. This is not too surprising since we previously observed that the carbene complexes of iron and ruthenium porphyrins are stable in water and protic solvents.[20]

The insertion of electrophilic carbenes in the N–H bonds of protected α-amino esters or amides is a powerful method for N-alkylating this class of compounds.[21] Since we[19,22] and others[23–25] previously reported N–H insertion of diazoesters catalyzed by metalloporphyrins (Fe, Ru), N–H insertion with a diazoketone such as DON was also investigated (Scheme 5). The results presented in Table 2 (entries 7 and 8) show that the ruthenium complex, and to



Scheme 4.

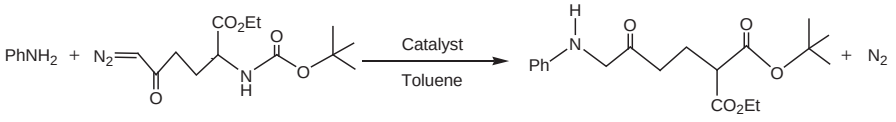
Table 2
S–H and N–H insertions with $N_2CHCO(CH_2)_2CH(CO_2Et)NHBoc$ catalyzed by Ru(CO)(TPP) (**1**) and Fe(Cl)(TPP) (**3**)[a]

Entry	Catalyst	Substrate	Product	Yield[b] (%)
1	**1**	PhSH	$PhSCH_2COCH_2CH_2CHRNHBoc$	85
2	**3**	PhSH	$PhSCH_2COCH_2CH_2CHRNHBoc$	51
3	**1**	$H_2C{=}CHCH_2SH$	$H_2C{=}CHCH_2SCH_2COCH_2CH_2CHRNHBoc$	87
4	**3**	$H_2C{=}CHCH_2SH$	$H_2C{=}CHCH_2SCH_2COCH_2CH_2CHRNHBoc$	58
5	**1**	$HOCH_2CH_2SH$	$HOCH_2CH_2SCH_2COCH_2CH_2CHRNHBoc$	70
6	**3**	$HOCH_2CH_2SH$	$HOCH_2CH_2SCH_2COCH_2CH_2CHRNHBoc$	54
7	**1**	$PhNH_2$	Ph NH $CH_2COCH_2CH_2CHRNHBoc$	71
8	**3**	PhNH2	Ph NH $CH_2COCH_2CH_2CHRNHBoc$	55

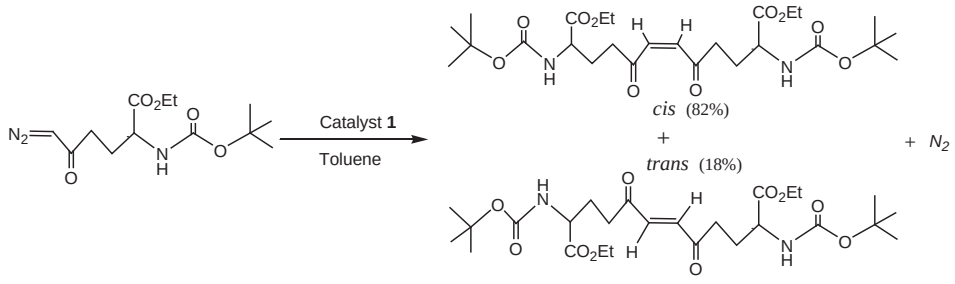
[a] A molar ratio of 1:200:200 for catalyst: diazo: substrate was employed at ambient temperature in toluene.
[b] Yields were determined by isolation of insertion product by column chromatography on silica gel.

a less extent the iron complex, are good catalysts for the transformation of aniline into the expected products. Due to a probable reduction of iron(III) to iron(II), addition of cobaltocene together with complex **3** was not necessary in the reaction. However the reaction needs 5 h to be completed compared to few minutes when diazoacetate was used as reagent.[19,24] The stereochemical constraints that result from the steric interaction of the axial carbene atoms with atoms of the porphyrinato core[26] may explain the decrease of the rate. The mechanisms of the cyclopropanation reaction, N–H insertion and S–H insertion, catalyzed by metalloporphyrins, have been previously discussed by us[19] and others[23–25] several times, and will not be presented herein.

Next, we also report that the ruthenium complex **1** catalyzes the stereoselective decomposition of protected DON to form olefinic products (Scheme 6). This coupling reaction catalyzed by ruthenium porphyrin to form olefins, proceeds through a metallocarbene intermediate (detected by the chemical shift of the carbene proton



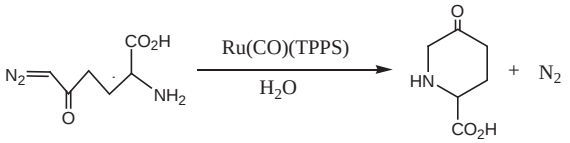
Scheme 5.



Scheme 6.

which appears at a very low field, 13.72 ppm by ^{1}H NMR), giving rise to a high cis/trans ratio of 82/18 in overall 81% yield. Attribution of cis and trans isomer compounds was realized by NMR (HMBC, see Experimental section). In comparison, a similar ruthenium(II) porphyrin species has been reported to catalyze rapidly the decomposition of ethyl diazoacetate to form diethyl maleate and diethyl fumarate with a cis/trans ratio of 15.[27] The generally accepted reaction pathway for transition metal catalyzed carbene dimerization of diazo derivatives involves initial attack of the diazo compound at the metal center to generate a metal carbene.[28,29] Nucleophilic attack on the metal carbene by diazo compound, followed by dissociation of the olefin from the metal complex, completes the catalytic cycle. The origin of cis selectivity during the coupling of α-diazo compounds has been previously interpreted on the basis of steric and electronic effects.[30–32]

Finally, reactivity of unprotected DON was also tested using ruthenium porphyrin **5** as a water-soluble catalyst (Scheme 7). Complex **5** in presence of an excess of unprotected 6-diazo-5-oxo-L-norleucine in water at 25 °C under an inert atmosphere gives, in 73% yield, 5-keto-L-pipecolic acid, resulting from an intramolecular N–H insertion. In the presence of 10 equiv of styrene in water, no cyclopropanation products are observed at room temperature and only intramolecular N–H insertion is noted. Although an N–H insertion was previously observed for the *N*-protected DON methyl ester catalyzed by rhodium acetate,[33,34] such reaction for unprotected DON is quite surprising owing the presence of a free acid group on the substrate. The present N–H insertion can offer a direct way to *cis*-5-hydroxy-L-pipecolic acid, a natural product, which is present in various plants such as acacia and Rhodesian teak,[33] from a commercially available but quite expensive diazo derivative.



Scheme 7.

3. Discussion

The antitumor diazoketone, 6-diazo-5-oxo-L-norleucine (DON), has been isolated from a natural source and can be considered as a modified α-amino acid. This glutamine analog 6-diazo-5-oxo-L-norleucine (DON) has been shown to bind and irreversibly inactivate glutaminase/asparaginases from several organisms at low concentration[3] and, to a less extent, also inactivate, glutaminase enzymes from the β-lactamase superfamily.[35] In the latter case, it appears that the enzyme removes the diazo group of DON, producing N_2 and 5-oxo-L-norleucine covalently bound to the serine 74 side-chain through its terminal carbon atom. Since several different functionalities, the diazoketone unit, the amino group and the carboxylic acid group, are in the same molecule, different reactions in biological media are possible such as intramolecular N–H insertion or intermolecular carbene transfers. A wide range of metal catalysts derived from copper, iron, and others have been reported to catalyze the diazo reagent decomposition[21,36,37] and, consequently, metalloproteins can be also considered as good candidates to react with DON.[20] Since evidences have been presented in favor of the formation of cytochrome P450-iron-carbene complexes during metabolism of various substrates,[38,39] the catalytic decomposition of DON have been carried out in the presence of metalloporphyrins as models of the active site of heme proteins. Accordingly, we herein discovered that intermolecular transfer of *N*- and *O*-protected 6-diazo-5-oxo-L-norleucine is possible. Three intermolecular insertion products have been identified from three different reactions giving the cyclopropane product, the S–H insertion product and the N–H insertion product, respectively, without any intramolecular reaction.

There have been a number of examples of $Rh_2(OAc)_4$-catalyzed intramolecular N–H insertions as a key step in the synthesis of β-lactams.[40,10] Most of the intramolecular N–H insertion reactions catalyzed by $Rh_2(OAc)_4$ involve insertion into amide bonds.[40] Consequently, intermolecular reactions with diazoketones derived from protected amino acids are difficult with rhodium acetate catalysts and consequently, of rather limited use. It should also be noted that addition of electrophilic carbenes to olefinic amino acid derivatives is also possible but this approach is limited due to a low diastereoselectivity.[21] In contrast, carbene transfers catalyzed by metalloporphyrins are much more selective[41,42] due probably to a lower reactivity of the metal-carbene intermediate.

To examine whether metalloporphyrins could be used for intramolecular N–H insertion, the diazo was completely deprotected. In this case, the intramolecular reaction was observed and the cyclic product was obtained in 71% yield using **5** as catalyst. In this case, intramolecular N–H insertion is probably much faster than intermolecular addition of the unprotected diazo derivative to the double bond of styrene, since no intermolecular addition was observed, even in presence of a large excess of styrene. It should be noted that we previously reported with a similar catalytic system[19] that N–H insertion is preferred to cyclopropanation reaction when 4-aminostyrene is the substrate. The intramolecular insertion led to 5-oxo-L-pipecolic acid, which is an intermediate in the synthesis of the natural product, *cis*-5-hydroxy-L-pipecolic acid, which is present in various plants such as Rhodesian teak, dates and acacia.[33,34]

4. Conclusion

In summary, the intermolecular carbene transfer of *N*- and *O*-protected DON catalyzed by iron or ruthenium porphyrin occurs in a highly selective manner giving cyclopropanation, N–H and S–H insertion reactions. It should be noted that no intramolecular N–H amide bond insertion was detected in contrast to reaction catalyzed by rhodium acetate. However, with unprotected DON, only intramolecular N–H insertion in the free amino group was observed, even in presence of a large excess of alkene. Investigation of the catalytic properties of these metalloporphyrins for the epoxidation of olefins in water is currently underway in our laboratory and will be reported in due course.

5. Experimental section

5.1. General experiments

All reactions were performed under argon and were magnetically stirred. Solvents were distilled from appropriate drying agent prior to use: toluene from sodium and benzophenone, CH_2Cl_2 from CaH_2, $CHCl_3$ from P_2O_5. Commercially available reagents were used without further purification unless otherwise stated. All reactions were monitored by TLC with Merck pre-coated aluminum foil sheets (Silica gel 60 with fluorescent indicator UV_{254}). Compounds were visualized with UV light at 254 nm and 365 nm. Column chromatographies were carried out using silica gel from Merck (0.063–0.200 mm). ^{1}H NMR and ^{13}C NMR in $CDCl_3$ were recorded using Bruker (Advance 500dpx and 300dpx spectrometers). UV–visible spectra were recorded on a UVIKON XL from Biotech. The enantiomeric excess of the cyclopropane was determined on a Varian Prostar 218 system equipped with Chiralcel columns.

The porphyrins were synthesised according to literature methods.[43] The corresponding ruthenium carbonyl complexes, Ru(CO)(TPP) and Ru(CO)(Halt), were obtained by refluxing the

porphyrins in *o*-dichlorobenzene with Ru_3CO_{12} at 180 °C[44,45] and the iron porphyrins were prepared as previously reported.[46]

5.2. Preparation[11] of $N_2CHCO(CH_2)_2CH(CO_2Et)NHBoc$ (Scheme 1)

5.2.1. Synthesis of (S)-(+)-ethyl-pyroglutamate. Freshly distilled thionyl chloride (30 ml) was added to a solution of L-glutamic acid (25.6 g, 175 mmol) in 250 ml of absolute ethanol cooled in a ice bath. The reaction mixture was stirred at room temperature for 1 h and heated at reflux for 0.5 h. This solution was neutralized with KOH in ethanol. The salt KCl formed was removed by suction filtration. After evaporation of the solvent, the product was purified by distillation under reduced pressure, yielding a colorless oil, which solidified at room temperature, 18 g (66%). ^{1}H NMR ($CDCl_3$, ppm) δ 1.24 (t, 3H, J=7 Hz), 2.27–2.47 (m, 4H), 4016 (q, 2H, J=7 Hz), 7.26 (br s, 1H).

5.2.2. Synthesis of (S)-(+)-ethyl-N-tert-butoxycarbonyl pyroglutamate. To a solution of (*S*)-(+)-5-carbethoxy-2-pyrrolidinone (2 g, 12.7 mmol), in 20 ml CH_2Cl_2 were added triethylamine (1.77 ml, 12.7 mmol), di-*tert*-butyl dicarbonate (5.56 g, 25.4 mmol), and 4-(dimethylamino) pyridine (1.55 g, 12.7 mmol). The solution was stirred for 7 h at 25 °C under an argon atmosphere. The solvent was removed, and the residue was purified by column chromatography on silica gel. Elution with dichloromethane/pentane/ether: 1/1/0.2 afforded 2.75 g (84%) of the desired compound. ^{1}H NMR ($CDCl_3$, ppm) δ 1.32 (t, 3H, J=7.0 Hz), 1.52 (s, 9H), 2.04–2.10 (m, 1H), 2.29–2.44 (m, 1H), 2.51–2.67 (m, 2H), 4.25 (q, 2H, J=7.0 Hz), 4.60, 4.64 (2d, 1H, J=2.2 Hz).

5.2.3. Synthesis of (S)-(+)-N-tert-butoxycarbonyl-6-diazo-5-oxo-norleucine ethyl ester. To a cold (−100 °C) solution of trimethylsilyldiazomethane (4.2 ml of a 2 M solution in hexane, 8.4 mmol) in THF (40 ml) under an inert atmosphere was added *n*-butyllithium in hexane (5.4 ml of a 1.6 M solution in hexane, 8.6 mmol). The reaction was stirred for 30 min and transferred via a cannula to a solution of (*S*)-(+)-5-carbethoxy-*N*-*tert*-butoxycarbonyl-2-pyrrolidone (7 mmol) in THF (70 ml) at −105 °C. The mixture was stirred for a further 10 min and quenched by addition of the cold solution to saturated ammonium chloride 200 ml. After extraction with ethyl acetate, the residue obtained after evaporation of solvents was purified by column chromatography on silica gel eluted with dichloromethane/pentane/ether: 1/1/0.2. After evaporation of solvents, the resulting yellow oil was dissolved in a minimum of ether. The product recrystallizes as pale yellow plates (1 g, 50%). ^{1}H NMR ($CDCl_3$, ppm) δ 1.26 (t, 3H, J=7.2 Hz), 1.46 (s, 9H), 2.07–1.92 (m, 1H), 2.12–2.29 (m, 1H), 2.44 (br t, 2H), 4.16–4.30 (q+m, 2H, J=7.0 Hz), 5.20 (br d, 1H, J=7.6 Hz), 5.31 (br s, 1H).

5.3. Preparation[11] of $N_2CHCO(CH_2)_2CH(CO_2H)NH_2$ (DON) (Scheme 2)

5.3.1. Synthesis of (S)-(+)-ethyl-N-Fmoc-pyroglutamate. To a solution of (*S*)-(+)-ethyl-pyroglutamate (300 mg, 1.91 mmol) in THF (10 ml) at −78 °C was added LHDMS (1.81 ml of a 1 M solution in THF, 1.81 mmol) slowly. The resultant pale yellow mixture was stirred at −78 °C for 15 min, and slowly transferred via a cannula to a solution of Fmoc-Cl (2.47 g, 9.55 mmol) in THF (10 ml) at −78 °C. The reaction was allowed to stir at −78 °C for 2 h, after which it was allowed to rise to room temperature. After 14 h of stirring at room temperature, the reaction was quenched by addition of saturated NH_4Cl (2 ml), and H_2O (1 ml). The solution was extracted with ethyl acetate and washed with brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluted with pentane/ethyl acetate (4/1). Recrystallization in hot ethyl acetate afforded 605 mg (83%) of the desired product as a colorless crystalline solid. ^{1}H NMR ($CDCl_3$, ppm) δ 1.30 (t, 3H, J=7.2 Hz, $CO_2CH_2CH_3$), 2.08–2.20, 2.32–2.52, 2.58–2.85 (3 m, 1H+1H+2H, CH_2CH_2), 4.20–4.37 (m, 3H, CO_2CH_2+CH), 4.47–4.72 (m, 3H, OCH_2+*CH), 7.31, 7.45 (2 t, 4H, J=7.4 Hz, mH Ph), 7.75, 7.80 (2d, 4H, J=7.4 Hz, oH Ph).

5.3.2. Synthesis of (S)-(+)-N-Fmoc -6-diazo-5-oxo-norleucine ethyl ester. To a cold (−100 °C) solution of trimethylsilyldiazomethane (3.15 ml of a 2 M solution in hexane, 6.31 mmol) in THF (30 ml) under an inert atmosphere was added *n*-butyllithium in hexane (4.04 ml of a 1.6 M solution in hexane, 6.48 mmol).The reaction was stirred for 30 min and transferred via a cannula to a solution of (*S*)-(+)-ethyl-*N*-Fmoc-pyroglutamate (2 g, 5.27 mmol) in THF (50 ml) at −105 °C. The mixture was stirred for a further 10 min and quenched by addition of the cold solution to saturated ammonium chloride 170 ml. After extraction with ethyl acetate, the residue obtained after evaporation of solvents was purified by column chromatography on silica gel eluted with pentane/ethyl acetate (7/3). After evaporation of solvents, the resulting yellow oil was dissolved in a minimum of ethyl acetate. The product recrystallizes as pale yellow plates (1.22 g, 55%). ^{1}H NMR ($CDCl_3$, ppm) δ 1.32 (t, 3H, J=7.2 Hz, $CO_2CH_2CH_3$), 2.00–2.44 (m, 4H, $COCH_2CH_2C^*$), 4.20–4.29 (m, 3H, CO_2CH_2+CH), 4.38–4.45(m, 3H, OCH_2+*CH), 5.29 (br s, 1H, CHN_2), 5.60 (br d, 1H, J=7.4 Hz, NH), 7.35, 7.44 (2 t, 4H, J=7.4 Hz, mH Ph), 7.63, 7.81 (2d, 4H, J=7.2 Hz, oH Ph).

5.3.3. Synthesis of (S)-(+)-6-diazo-5-oxo-norleucine (DON). (*S*)-(+)-*N*-Fmoc-6-diazo-5-oxo-norleucine (1 g, 2.37 mmol) was added to piperidine (20 ml) and the mixture stirred for 2 min. The resulting solution was poured into ice-cold water (60 ml). The reaction was filtered and the filtrate evaporated under high vacuum at room temperature. The pale brown residue was crystallized from a minimum amount of water by addition of methanol at −18 °C to give 210 mg (52%) of the desired product as a pale yellow solid. ^{1}H NMR (D_2O, ppm) δ 2.08–2.15 (m, 2H, CH_2C^*), 2.52 (br t, 2H, J=7 Hz, CH_2CO), 3.73 (t, 1H, J=6.2 Hz, C^*H), 5.87 (br s, 1H, CHN_2).

5.4. Cyclopropanation of styrene using catalyst 1, 2, 3, 4 with $N_2CHCO(CH_2)_2$ CH (CO_2Et) NHBoc (Scheme 3)

In a typical experiment 1 μmol of catalyst and 1 mmol of styrene were placed in a Schlenk tube under argon, and dissolved in 400 μl of toluene. With the Fe-porphyrin catalysts **3** and **4**, 2 mg (10 μmol) of cobaltocene was added for the reduction of Fe. The diazo compound (0.2 mmol in 100 μl of toluene) was then slowly added at room temperature. After 5 h of stirring, the cyclopropanes were purified by column chromatography on silica gel (pentane/CH_2Cl_2/ether: 4/5/1) to give a mixture of *cis* and *trans*-2-phenyl-1-5-oxo-*N*-Boc-L-norleucine ethyl ester cyclopropanes. The trans/cis diastereoselectivity was determined by ^{1}H NMR. The dimer product was then recovered after elution with CH_2Cl_2.

The ee was determined by chiral HPLC analysis using a Chiralcel OD column: *n*-hexane/^{i}PrOH=90/10, flow rate=0.5 mL/min, wavelength=250 nm:

Cyclopropanation catalyzed by (−) **2**: t_r=21.12 min for the minor isomer, t_r=25.32 min for the major isomer, 79.50% ee, $[\alpha]_D^{25}$ −168.5 (*c* 0.27, CH_2Cl_2).

Cyclopropanation catalyzed by (+) **2**: t_r=20.57 min for the major isomer, 68.20% ee, t_r=25.70 min for the minor isomer, $[\alpha]_D^{25}$ +128 (*c* 0.27, CH_2Cl_2).

Cyclopropanation catalyzed by (−) **4**: t_r=20.93 min for the minor isomer, t_r=24.79 min for the major isomer, 79.80% ee, $[\alpha]_D^{25}$ −170 (*c* 0.35, CH_2Cl_2).

^{1}H NMR ($CDCl_3$, ppm), trans isomer: δ 1.29 (t, J=7.1 Hz, 3H, $CO_2CH_2CH_3$), 1.38–1.42 (m, 1H, CH_2 cyclopropane), 1.45 (s, 9H, *tert*-butyl), 1.67–1.72 (m, 1H, CH_2 cyclopropane), 1.92–1.98 (m, 1H, CH_2C^*), 2.17–2.22 (m, 2H, CH_2C^*+CHPh cyclopropane), 2.51–2.56 (m, 1H, CH cyclopropane), 2.70–2.76 (m, 2H, CH_2CO), 4.21 (q, J=7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.27–4.32 (m, 1H, $^*CHCO_2Et$), 5.13 (br d, J=6.7 Hz, 1H, NH), 7.11 (d, J=7.4 Hz, 2H, oPh), 7.24 (t, J=6.7 Hz, 1H, pPh), 7.31 (t, J=7.4 Hz, 2H, mPh). ^{13}C NMR ($CDCl_3$, ppm) and HMQC δ 14.17 CH_3 (CO_2Et), 19.14 (CH_2 cyclopropane), 28.60 (CH_2C^*), 28.31 (CH_3 *tert*-butyl), 29.07 (CH cyclopropane), 32.36 (CH Ph), 39.69 (CH_2CO), 53.02 ($^*CH$), 61.48 CH_2 (CO_2Et), 79.92 (C *tert*-butyl), 126.03 (oPh), 126.56 (pPh), 128.50 (mPh), 140.22 (*Cipso*Ph), 155.46 (CONH), 172.39 (CO_2Et), 207.58 (C=O). HRMS (ESI): calcd for $C_{21}H_{29}NO_5$ $(M+Na)^+$: 398.19434, found: 398.1943.

5.5. S–H insertion with $N_2CHCO(CH_2)_2CH(CO_2Et)NHBoc$ catalyzed by Ru(CO)(TPP) (1) and Fe(Cl)(TPP) (3) (Scheme 4)

In a typical experiment, 1 μmol of catalyst was placed in a Schlenk tube under argon, and dissolved in 400 μl of toluene. With the Fe-porphyrin catalysts **3** and **4**, 2 mg (10 μmol) of cobaltocene was added for the reduction of Fe. Thioanisole (0.2 mmol) and diazo (0.2 mmol) in 100 μl of toluene were then slowly added at room temperature. After 15 h of stirring, the insertion product was purified by column chromatography on silica gel (pentane/CH_2Cl_2/ether:1/1/0.1).

5.5.1. 5-Oxo-N-Boc-L-norleucine ethyl ester phenylsulfide. ^{1}H NMR ($CDCl_3$, ppm) δ 1.30 (t, J=7.0 Hz, 3H, $CO_2CH_2CH_3$), 1.47 (s, 9H, *tert*-butyl), 1.81–1.94 (m, 1H, CH_2C^*), 2.06–2.21 (m, 1H, CH_2C^*),2.69–2.75 (m, 2H, CH_2CO), 3.72 (s, 2H, $SHCH_2CO$), 4.21 (q, J=7.0 Hz, 2H, $CO_2CH_2CH_3$), 4.29–4.34 (m, 1H, $^*CHCO_2Et$), 5.12 (br d, J=7.2 Hz, 1H, NHCO),7.28–7.35(m, 5H, Ph). ^{13}C NMR and HMQC ($CDCl_3$, ppm) δ 14.14 (CH_3 (CO_2Et)), 26.82(CH_2C^*), 28.29 (CH_3 *tert*-butyl), 36.41 (CH_2CO),43.95 (CH_2S), 52.78 ($^*CH$), 61.52 (CH_2 (CO_2Et)), 79.98 (C *tert*-butyl), 128.77 (pPh), 129.16 (mPh), 129.52 (oPh), 134.70 (*Cipso*Ph), 156.40 (CONH), 172.04 (CO_2Et), 204.51 (C=O). HRMS (ESI): calcd for $C_{19}H_{27}NO_5S$ $(M+Na)^+$: 404.1508, found: 404.1509.

5.5.2. 5-Oxo-N-Boc-L-norleucine ethyl ester allyl sulfide. ^{1}H NMR ($CDCl_3$, ppm) δ 1.32 (t, J=7.0 Hz, 3H, $CO_2CH_2CH_3$), 1.47 (s, 9H, *tert*-butyl), 1.87–1.98 (m, 1H, CH_2C^*), 2.14–2.21 (m, 1H, CH_2C^*), 2.71–2.78 (m, 2H, CH_2CO), 3.14 (d, J=7.4 Hz, 2H, CH_2S), 3.22 (s, 2H, SCH_2CO), 4.23 (q, J=7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.29–4.34 (m, 1H, $^*CHCO_2Et$), 5.14 (br d, J=7.2 Hz, 1H, NHCO), 5.20 (m, 2H, CH_2 allyl), 5.69–5.83 (m, 1H, CH allyl). ^{13}C NMR ($CDCl_3$, 125 MHz) and HMQC δ 14.15 (CH_3 (CO_2Et)), 26.75(CH_2C^*), 28.28 (CH_3 *tert*-butyl), 34.65 (CH_2S), 36.51 (CH_2CO), 52.90 ($^*CH$), 61.51 (CH_2 (CO_2Et)), 79.96 (C *tert*-butyl), 118.49 (CH_2 allyl), 132.82 (CH allyl), 156.40(CONH), 172.31 (CO_2Et), 204.73 (C=O). HRMS (ESI): calcd for $C_{16}H_{27}NO_5S$ $(M+Na)^+$: 368.1508, found: 368.1511.

5.5.3. 5-Oxo-N-Boc-L-norleucine ethyl ester ethan-2-ol sulfide. ^{1}H NMR ($CDCl_3$, ppm) δ 1.29 (t, J=7.3 Hz, 3H, $CO_2CH_2CH_3$), 1.45 (s, 9H, *tert*-butyl), 1.79–1.89 (m, 1H, CH_2C^*), 2.15–2.22 (m, 1H, CH_2C^*), 2.74–2.82 (m, 2H, CH_2CO), 2.72 (t, J=5.8 Hz, 2H, CH_2S), 3.31 (s, 2H, SCH_2CO), 3.75 (t, 2H, J=5.7 Hz, CH_2OH), 4.22 (q, J=7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.24–4.29 (m, 1H, $^*CHCO_2Et$), 5.16 (br d, J=6.1 Hz, 1H, NHCO). HRMS (ESI): calcd for $C_{15}H_{27}NO_6S$ $(M+Na)^+$: 372.14568, found: 372.1456.

5.6. N–H insertion of aniline with $N_2CHCO(CH_2)_2CH(CO_2Et)$ NHBoc catalyzed by Ru(CO)(TPP) (1) and Fe(Cl)(TPP) (3) (Scheme 5)

In a typical experiment 1 μmol of catalyst was placed in a Schlenk tube under argon, and dissolved in 400 μl of toluene. Aniline (0.2 mmol) and diazo (0.2 mmol) in 100 μl of toluene were then slowly added at room temperature. After 5 h of stirring, the insertion product was purified by column chromatography on silica gel (pentane/CH_2Cl_2/ether: 2/7/1) to give *N*-5-oxo-*N*-Boc-L-norleucine ethyl ester phenyl amine. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, J=7.0 Hz, 3H, $CO_2CH_2CH_3$), 1.46 (s, 9H, *tert*-butyl), 1.93–2.03 (m, 1H, CH_2C^*), 2.22–2.31 (m, 1H, CH_2C^*), 2.61–2.71 (m, 2H, CH_2CO), 4.03 (s, 2H, $NHCH_2CO$), 4.23 (q, J=7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.29–4.34 (m, 1H, $^*CHCO_2Et$), 4.60 (br s, 1H, NHPh), 5.15 (br d, J=7 Hz, 1H, NHCO), 6.62 (d, J=8.4 Hz, 2H, oPh), 6.71 (t, J=7.4 Hz, 1H, pPh), 7.23 (t, J=8.0 Hz, 2H, mPh). ^{13}C NMR ($CDCl_3$, 125 MHz) and HMQC δ 14.13 (CH_3 (CO_2Et)), 27.65 (CH_2C^*), 28.30 (CH_3 *tert*-butyl), 37.47 (CH_2CO), 49.59 (CH_2NH), 53.42 ($^*CH$), 61.48 (CH_2 (CO_2Et)), 80.00 (C *tert*-butyl), 112.92 (oPh), 117.55 (pPh), 129.34 (mPh), 146.62 (*Cipso*Ph), 155.77(CONH), 171.91 (CO_2Et), 206.16 (C=O). HRMS (ESI): calcd for $C_{19}H_{28}N_2O_5$ $(M+Na)^+$: 387.1896, found: 387.1896.

5.7. Dimerization of $N_2CHCO(CH_2)_2CH(CO_2Et)NHBoc$ catalyzed by Ru(CO)(TPP) (1) (Scheme 6)

Ru(CO)(TPP) (0.75 mg, 1 μmol) was dissolved in 500 μl of toluene in a Schlenk tube under argon. Diazo (30 mg, 0.10 mmol) in 200 μl of toluene was then added slowly at room temperature and stirred for 2 h. The dimerization product was purified by column chromatography on silica gel (pentane/CH_2Cl_2/ether: 0.1/0.7/0.2) to give 22 mg (yield=81%) of the dimer compound. In the ^{1}H NMR spectrum, the proportions of the cis and trans dimer compounds were, respectively 82 and 18%. The attribution of cis and trans isomer compounds was realized by HMBC ($CDCl_3$, ppm) δ (^{13}C) 201.42 and δ (^{1}H) 6.32 (J_{C1H2}=7.5 Hz, O=C_1–CH_1=CH_2, *cis*), δ (^{13}C) 198.89 and δ (^{1}H) 6.79 (J_{C1H2}=4.9 Hz, O=C_1–CH_1=CH_2, *trans*). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.29 (t, J=7.0 Hz, 3H, $CO_2CH_2CH_3$), 1.45 (s, 9H, *tert*-butyl), 1.92–2.03 (m, 1H, CH_2C^*), 2.19–2.27 (m, 1H, CH_2C^*),2.67 (dd, J=8.7, 8.1 Hz, 2H, CH_2CO), 4.22 (q, J=7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.25–4.31 (m, 1H, $^*CHCO_2Et$), 5.12 (br d, J=7.2 Hz, 1H, NHCO), 6.32 (s, 2H, *cis* CH=CH), 6.79 (s, 2H, *trans* CH=CH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.15 (CH_3 (CO_2Et), 26.45(CH_2C^*), 28.30 (CH_3 *tert*-butyl), 38.35 (CH_2CO)), 52.84 ($^*CH$), 61.53 (CH_2 (CO_2Et)), 79.95 (C *tert*-butyl), 135.62 (CH=CH), 155.51(CONH), 172.25 (CO_2Et), 201.42 (C=O). HRMS (ESI): calcd for $C_{26}H_{42}N_2O_{10}$ $(M+Na)^+$: 565.27372, found: 565.2733.

5.8. N–H insertion of (*S*)-(+)-6-diazo-5-oxo-norleucine (DON) catalyzed by Ru(CO)(TPPS) (5) (Scheme 7)

A solution of the diazoketone DON (34.2 mg, 200 μmol) in degazed water (0.5 ml) was added dropwise for 1 h to a solution of Ru(CO)(TPPS) (1.2 mg, 1 μmol) in 0.5 ml degazed water. After 5 h of stirring, evaporation of the water gave 21 mg (73%) of the insertion product as a solid. ^{1}H NMR (D_2O, ppm) δ 1.88–2.00 (m, 3H, CH_2CH_2), 2.21–2.23 (m, 1H, CH_2CH_2), 3.07, 3.27 (2d, 2H, CH_2), 3.67–3.71 (m, 1H, HC^*), 3.88 (br s, 1H, NH). HRMS (ESI): calcd for $C_6H_8NO_3$ $(M-H)^-$: 142.05097, found: 142.0511. $[\alpha]_D^{25}$ −24 (*c* 0.66, D_2O).

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