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# A new reagent for the introduction of Boc protecting group to amines: Boc-OASUD

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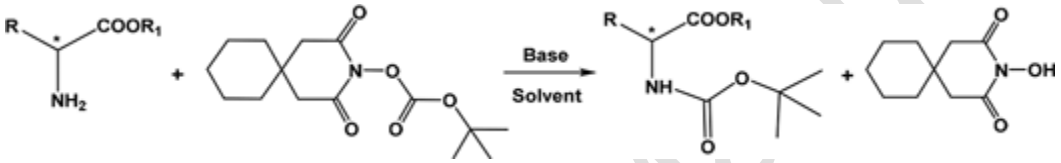
**ABSTRACT**

A new reagent, *tert*-butyl (2, 4-dioxo-3-azaspiro [5,5] undecan-3-yl) carbonate (Boc-OASUD) for the preparation of N-Boc-amino acids is described. The Boc-OASUD reacts with amino acids and their esters at room temperature in the presence of a base and gives N-Boc-amino acids and their esters in good yields and purity. Introduction of the Boc group takes place without racemization. The Boc-OASUD, being a solid and more stable, is a better alternative to di-*tert*-butyl dicarbonate which is low melting and has to be dispensed in plastic containers than glass because of its poor stability.

**GRAPHICAL ABSTRACT**




**KEYWORDS:** amino acids, Boc-OASUD, HO-ASUD, protecting group

Di-*tert*-butyl dicarbonate ($Boc_2O$) is the most widely used reagent for the introduction of the *tert*-butoxycarbonyl (Boc) group for the protection of the amino group in the peptide synthesis.[1] However, the main drawback of $Boc_2O$ is its low melting range of 22-24$^oC$, making it a liquid in most geographical regions and seasons. It tends to decompose on storage. Bottles of $Boc_2O$ build internal pressure in sealed containers caused by its slow decomposition to *tert*-butanol and $CO_2$ in the presence of moisture. For this reason, it is usually sold and stored in plastic bottles rather than glass ones.[2] The inhalational toxicity of $Boc_2O$ is also high and is comparable to that of phosgene.[2] Thus there is a need for an alternative reagent which is solid and more stable for the introduction of Boc group.

Recently, we reported N-hydroxy-3-azaspiro[5,5]undecane-2,4-dione (HO-ASUD **1**), as an useful condensing agent, for preparing peptides.[3] Based on **1**, we also reported, Fmoc-OASUD, as a novel reagent for the introduction of Fmoc group.[4]

Here, we describe *tert*-butyl (2, 4-dioxo-3-azaspiro [5,5] undecan-3-yl) carbonate (Boc-OASUD **2**) as a new Boc reagent for preparing N-Boc-amino acids (Scheme 1). The **2** is a crystalline solid exhibiting better stability and is a good alternative for $Boc_2O$.

## Results and Discussion

The reagent, **2** is prepared conveniently by reacting HO-ASUD[3] with $Boc_2O$ in a suitable solvent such as acetonitrile in the presence of a base such as triethylamine (Scheme 2). The reaction results in a colorless crystalline **2** in high yields (>85%) and purity (>99.5% HPLC).The reagent is soluble in most organic solvents such as methanol, ethanol, 2-propanol, tert-butanol, ethyl acetate, methyl tert-butyl ether, toluene, chloroform, acetonitrile, acetone, 1,4-dioxane, THF, 2-MeTHF, DMF and DMSO. It is insoluble in petroleum ether, n- Hexane, cyclohexane and water.

The reagent was found to be stable when stored at room temperature (25±3$^{o}$C) protected from air and moisture. After nine months storage, slight degradation (<0.5%) was observed and the main degradation product was **1**. The reagent was also found to be stable when kept at 40 $^{o}$C and 70 $^{o}$C for 24 hours (99.9% and 99.7% HPLC respectively). However at 90 $^{o}$C, it melted (mp: 85-88°C) and decomposed completely to OH-ASUD, **1** (97.8% HPLC).

The reagent **2** has high reactivity towards the aliphatic amino group under mild conditions. A number of N-Boc-amino acids were prepared by reacting amino acids with **2** in the presence of a base at room temperature in high yields and purity (**Table 1**). The amino acid is

dissolved in an aqueous solution containing a base such as sodium carbonate and reacted with a solution of **2** dissolved in a suitable water miscible solvent such as acetone at room temperature. Depending on amino acid, the reaction is completed in 9 to 18 hours (TLC). After the reaction, the pH was adjusted to 6 with $KHSO_4$ and washed with an organic solvent such as ethyl acetate to remove the liberated **1** and unreacted **2**. The **1** can be recovered again and converted back to **2** by evaporating the organic layer and by reacting the crude obtained with fresh $Boc_2O$ and triethylamine. The aqueous solution was further acidified using $KHSO_4$ to pH 2 to 4 and extracted with ethyl acetate. Evaporation of the solvent and recrystallization of the residue from ethyl acetate and hexane results in N-Boc-amino acid. In the case of Lysine and Valine, the corresponding Boc amino acids were isolated as their dicyclohexylamine (DCHA) salts. The H-Lys(Boc)-OH (*$N^{\epsilon}$-Boc-lysine*, entry 12), was prepared using lysine copper complex to block the alpha amino group. The copper complex was reacted with the reagent **2**, followed by cleaving the complex using EDTA resulted in the H-Lys (Boc)-OH.

All the N-Boc-derivatives were obtained in good yields and purity. During the reaction, the chiral integrity is maintained. In addition to sodium carbonate, other bases such as sodium hydroxide, or organic bases such as triethyl amine can also be used.

Apart from room temperature, the reaction was also studied at 50 (±2) $^oC$. In the case of L-Phenyl alanine, at both the temperatures, similar yields and purity were obtained. However in the case of L-Serine, racemization was observed at 50 (±2) $^oC$ (Chiral purity: 92.93 Vs 99.8%).

For comparison, we prepared N-Boc-L-Phe-OH and N-Boc-L-Ala-OH using both the **2** and $Boc_2O$. Reacting L-Phenyl alanine with **2** in acetone using $Na_2CO_3$ for 9 hours at 25 (±2) $^oC$ resulted in N-Boc-L-Phe-OH in 95% yield (99.9% HPLC). Reacting with $Boc_2O$ under same conditions resulted in 93% yield (99.9% HPLC). Similarly, reaction of L-Alanine with **2** in

acetonitrile using $Na_2CO_3$ for 10 hours at 25 (±2) $^oC$ resulted in N-Boc-L-Ala-OH in 92% yield (99.2% HPLC) and with $Boc_2O$ the yield was 96% (98.6% HPLC). Thus both reagents gave similar yields and purity.

The reagent was found to be specific to the amino group without reacting with either phenolic or aliphatic hydroxyl group when one equivalent of **2** was used. When L-Ser was reacted with 1.5 and two equivalents of **2**, about 0.39% and 1.98% of N-Boc-Ser (OBoc)-OH was observed respectively. Similarly, about 0.52% and 1.92% of N-Boc-Tyr (OBoc)-OH was observed respectively.

It is always a challenge to prepare N-Boc amino acid ester from the corresponding ester because of the possibility of ester hydrolysis in an alkaline aqueous medium. However, by using triethylamine as a base and reacting the ester with reagent **2**, in a non-aqueous organic solvent such as dichloromethane, a number of N-Boc-amino acid esters have been prepared (**Table 2**). Here also, in all the cases, good yields have been obtained without any racemization.

## Conclusion

In conclusion, we have described *tert*-butyl (2, 4-dioxo-3-azaspiro [5,5] undecan-3-yl) carbonate (Boc-OASUD **2**) as a novel reagent for the preparation of N-Boc protected amino acids and their esters. The new reagent is a stable crystalline material and reacts with the amino group in a facile manner under mild conditions without causing racemization.

## Experimental

### *Analytical methods*

All chemicals used were obtained from commercial sources and used without further purification. TLC was performed on Merck 60 F254 Silicagel plates Visualization was accomplished with UV light (254 nm) and ninhydrin. Melting points were determined in open capillaries on a Polmon melting point apparatus (Model No: MP-96) and are uncorrected. Optical rotations were measured with a Jasco DIP-1000 polarimeter. FTIR spectra were obtained from Perkin-Elmer Spectrum one, Spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker instrument at 300 and 75 MHz, respectively, with $CDCl_3$, $CD_3OD$ or DMSO-$d_6$ as solvent and relative to TMS as internal standard. Mass spectra were recorded on a Thermo scientific LCQ Fleet spectrometer with ion trap mass spectrometer. Column chromatography was conducted over silica gel (200-400 mesh).

### *Preparation of tert-butyl (2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl) carbonate (Boc-OASUD, 2)*

N-Hydroxy-3-azaspiro[5,5]undecane-2,4-dione (HO-ASUD) (5.0 g, 25.3 mmol), and triethyl amine (3.08 g, 30.4 mmol) were dissolved in acetonitrile (50 mL). To this solution, was added $Boc_2O$ (6.63 g, 30.4 mmol) at 0-5°C and stirred at 25-30°C for 5h. The reaction mixture was concentrated under reduced pressure and the crude material was dissolved in ethyl acetate (50 mL), washed with water (25 mL) and brine solution (25mL). The ethyl acetate layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The resulting material was precipitated with hexane (50 mL) to yield Boc-OASUD as a color less solid (6.50 g, 86.3% yield). mp: 85-88°C; Purity: 99.86% (by HPLC analysis: XTerra RP 18 (250 x 4.6 mm) column, 5 µm; eluent: water/acetonitrile (30:70); flow rate 1.0 mL/min; Temp 27°C, detection at 210 nm]. IR (KBr, $cm^{-1}$): $\nu_{max}$ 3445, 3008, 2980, 2927, 2848, 2860, 1790, 1754, 1713, 1454, 1428,

1412, 1397, 1369, 1349, 1339, 1273, 1244, 1196, 1158, 1142, 1115, 1080, 1052, 982, 963, 944, 924, 904, 894, 874, 805, 768, 718, 642, 608, 566, 542; $^{1}$H NMR (300 MHz, $CDCl_3$): δ 2.75-2.66 (m, 4H), 1.57-1.49 (m, 19H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 166.01, 150.19, 86.63, 44.10, 36.78, 34.88, 32.53, 27.48, 25.55, 21.45; MS: m/z 319.75 $[M+Na]^+$.

### *Preparation of N-(tert-Butoxycarbonyl)-L-phenylalanine (Table 1, entry 1)*

L-Phenylalanine (1.0 g, 6.05 mmol) and sodium carbonate (0.71 g, 6.70 mmol) in water (10 mL) was reacted with a solution of **2** (1.89 g, 6.36 mmol) in acetone (10 mL). The reaction mixture was stirred at room temperature till the reaction completes, as monitored by TLC. The resulting solution was concentrated, to the residue was added water (10 mL) and ethyl acetate (10 mL), pH adjusted to 6.0 with 10% $KHSO_4$ and stirred for 5 minutes. The organic layer was removed and the aqueous layer was acidified to pH 2.0 with 10% $KHSO_4$ at 0-5°C and extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with 5% $NaHCO_3$ solution, water, brine and dried over anhydrous $Na_2SO_4$. The ethyl acetate layer was concentrated and the residue was crystallized from EtOAc/hexane (2:8) mixture to give the product as a white solid (1.53g, 95% yield). mp: 85-87°C (lit.[5] 86-88°C); $[\alpha]_D^{20} = +25.9^0$ (*c* 1 in EtOH) {lit.[5] $[\alpha]_D^{20} = +25.5^0$ (*c* 1 in EtOH)}; 99.88% purity by HPLC [Method: XTerra RP 18 (250 x 4.6 mm) column, 5 µm; eluent: water/acetonitrile/glacial AcOH (65:35:0.1%); flow rate 1.0 mL/min; Temp 27°C, detection at 210 nm]; % ee: 99.76% (D-isomer R*t* = 6.26 min; L-isomer R*t* = 12.40 min) [Method: Chiralpak IA, 250 x 4.6 mm, 5 µm; eluent: n-Hexane: IPA: TFA (90:10:0.1%); flow rate 1.2 mL/min; Temp 27°C, detection: 215 nm]; IR (KBr, $cm^{-1}$): $\nu_{max}$ 3373, 3028, 2984, 2935, 1724, 1708, 1692, 1525, 1442, 1421, 1392, 1366, 1342, 1297, 1269, 1252, 1168, 1082, 1053, 1028, 1003, 966, 949, 888, 853, 789, 758, 748, 701; $^{1}$H NMR (300

MHz, DMSO-$d_6$): δ 12.59 (br, 1H), 7.30-7.16 (m, 5H), 7.11-7.09 (d, 1H), 4.12-4.04 (m, 1H), 3.04-2.98 (dd, 1H), 2.85-2.77 (m, 1H), 1.31 (s, 6H) and 1.25 (s, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 173.55, 155.39, 137.97, 129.03, 128.06, 126.25, 77.98, 55.08, 36.45, 28.01,27.78; MS: m/z 288.88 $[M+Na]^+$.

The other amino acids given in **Table 1** were prepared and characterized in a similar manner.

### *Preparation of methyl (tert-butoxycarbonyl)-L-phenylalaninate (Table 2, entry 1)*

L-Phenylalanine methyl ester hydrochloride (5.0 g, 23.2 mmol), triethyl amine (2.47 g, 24.4 mmol) and **2** (7.24 g, 24.4 mmol) were added to dichloromethane (50 mL) and stirred at reflux temp (38-40°C) for 5h. After completion of the reaction, filtered to remove salts and the filtrate was washed with 5% $KHSO_4$ (20 mL), water (25 mL), brine (25 mL), and dried over sodium sulfate. The solvent was evaporated under reduced pressure to obtain a pale yellow oil. The oil was purified by column chromatography (silica gel, n-hexane/ ethyl acetate, 8:2) to afford 5.96 g (92%) Methyl (*tert*-butoxycarbonyl)-L-phenylalaninate as a colorless oil. $R_f$ 0.5 (n-Hexane: EtOAc-4:1); $[\alpha]_D^{25}$ = -4.5$^0$ (*c* 1 in MeOH) {lit.[6] $[\alpha]_D^{25}$ = -6.0$^0$ (*c* 2.5, MeOH)}; IR (neat, cm$^{-1}$): $\nu_{max}$ 3437, 3020, 2980, 2954, 1735, 1719, 1604, 1507, 1492, 1454, 1444, 1437, 1391, 1364, 1250, 1177, 1154, 1079, 1053, 1015, 951, 932, 858, 817, 779, 739, 701, 668; $^1$H NMR (300 MHz, $CDCl_3$): δ 7.32-7.11 (m, 5H), 4.98-4.95 (d, 1H), 4.62-4.55 (q, 1H), 3.71 (s, 3H), 3.15-3.01 (m, 2H), 1.41 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 172.33, 155.08, 136.05, 129.28, 128.53, 127.00, 79.86, 54.44, 52.15, 38.34, 28.28; MS: m/z 302.90 $[M+Na]^+$.

The other N-Boc- amino acid esters given in **Table 2** were prepared and characterized in a similar manner.

**Supplemental Material**

Complete experimental and spectral details are available online in the Supplemental Material.

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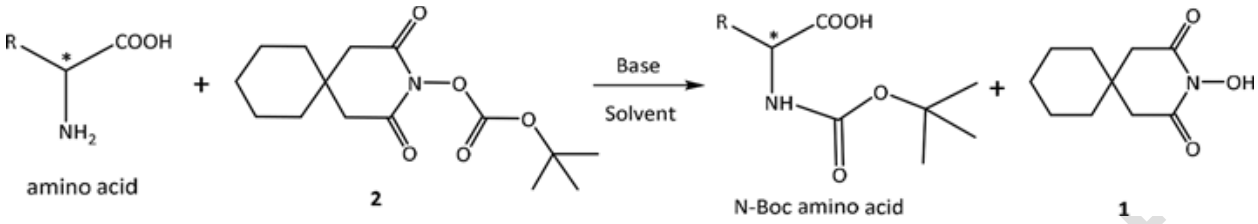
**Table 1.** Synthesis of N-Boc protected amino acids with 2[a].




| Entry | N-Boc Amino acid | L/D | Base | Solvent | Time (h) | Yield (%) |
|---|---|---|---|---|---|---|
| 1 | Boc-Phe-OH | L | $Na_2CO_3$ | acetone | 9 | 95 |
| 2 | Boc-Ala-OH | L | $Na_2CO_3$ | acetonitrile | 10 | 92 |
| 3 | Boc-Gly-OH | – | NaOH | acetone | 12 | 94 |
| 4 | Boc-Leu-OH | L | $Na_2CO_3$ | 1, 4-dioxane | 15 | 88 |
| 5 | Boc-Trp-OH | L | Triethyl amine | acetonitrile | 15 | 91 |
| 6 | Boc-Pro-OH | L | Triethyl amine | acetone | 12 | 86 |
| 7 | Boc-Tyr-OH | L | $Na_2CO_3$ | acetonitrile | 9 | 91 |
| 8 | Boc-Lys (Boc)-OH[b] | L | NaOH | 1, 4-dioxane | 18 | 81 |
| 9 | Boc-Val-OH[b] | L | $Na_2CO_3$ | acetone | 14 | 90 |
| 10 | 4-Cl-Boc-Phe-OH | D | Triethyl amine | acetone | 14 | 90 |
| 11 | 3-F-Boc-Phe-OH | L | $Na_2CO_3$ | acetonitrile | 12 | 85 |
| 12 | H-Lys (Boc)-OH[c] | L | $Na_2CO_3$ | acetone | 18 | 78 |

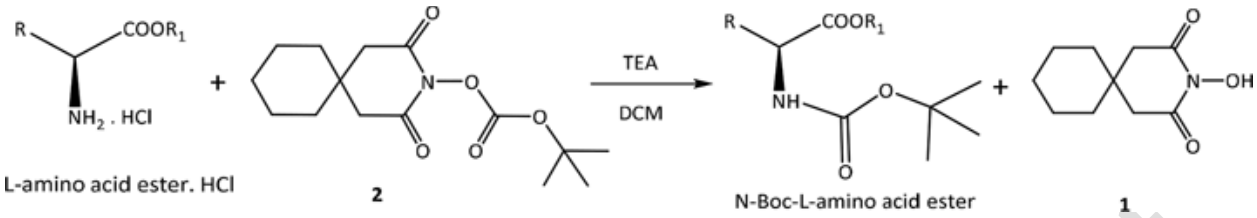
| | | | | | | |
|---|---|---|---|---|---|---|
| 13 | Boc-Asp-OH | L | NaOH | acetonitrile | 15 | 80 |
| 14 | Boc-Phg-OH | D | $Na_2CO_3$ | 1, 4-dioxane | 12 | 89 |
| 15 | Boc-Ser-OH | D | Triethyl amine | acetone | 11 | 89 |

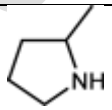
[a]Experimental conditions: The amino acid is dissolved in an aqueous solution containing a base and reacted with a solution of **2** dissolved in a suitable solvent at room temperature. After the reaction, the base was neutralized, extracted with an organic solvent. Evaporation followed by crystallization from EtOAc/hexane gives the required N-Boc amino acids. All products were identified by comparison with their spectral data (FTIR, $^{1}H$ & $^{13}C$NMR and MASS).

[b]The Boc-Lys (Boc)-OH (entry.8) and Boc-Val-OH (entry. 9) were isolated as a DCHA salt.

[c]The H-Lys (Boc)-OH (entry. 12) was prepared using copper complex method.

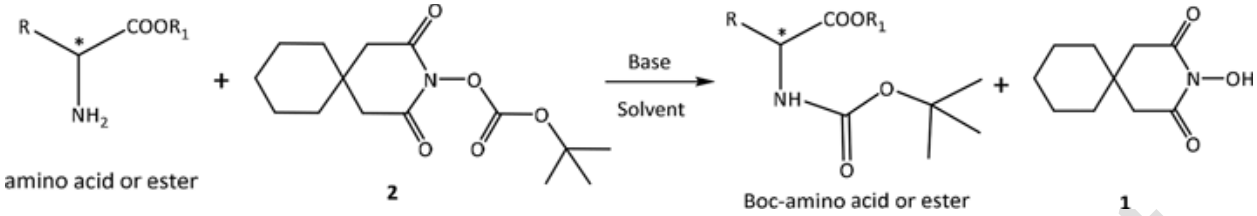
**Table 2.** Synthesis of N-Boc amino acid esters with 2*.




| Entry | R | $R_1$ | Yield (%) |
|---|---|---|---|
| 1 | $PhCH_2$ | Me | 92 |
| 2 | $CH_3$ | Me | 89 |
| 3 | $CH_3$ | Et | 85 |
| 4 | H | Me | 93 |
| 5 | H | Et | 91 |
| 6 | H | Bn | 90 |
| 7 | $CH_2OH$ | Me | 81 |
| 8 | $CH_2OH$ | Bn | 86 |
| 9 | $(CH_3)_2CH$ | Me | 87 |
| 10 |  | Me | 85 |
| 11 | 4-OH-$PhCH_2$ | Me | 82 |
| 12 | 4-OH-$PhCH_2$ | Et | 84 |

*L-amino acid ester hydrochloride (23.2 mmol), triethyl amine (24.4 mmol) and **2** (24.4 mmol) were added to dichloromethane (50 mL). The resulting mixture was refluxed for 3–5 h. The reaction mixture was filtered and concentrated under reduced pressure. The obtained residue was purified using column chromatography. All products were identified by comparison with their spectral data (FTIR, $^{1}H$ & $^{13}CNMR$ and MASS).

**Scheme 1.** Synthesis of N-Boc protected amino acids and esters using Boc-OASUD.



**Scheme 2.** Synthesis of Boc-OASUD.