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Synthesis of sydnonimines from sydnones and their use for bioorthogonal release of isocyanates in cells†

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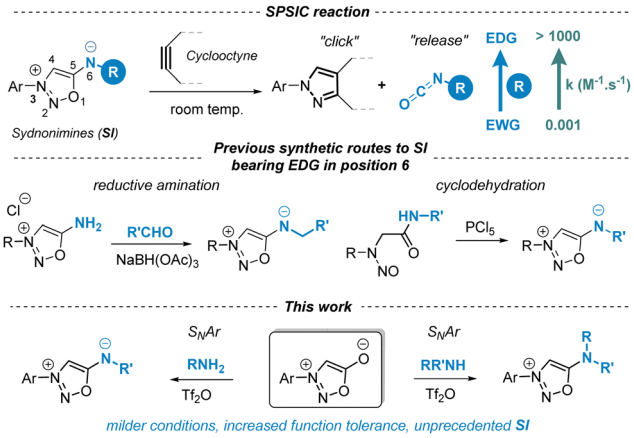
In this article, we report the synthesis of sydnonimines from sydnones and their use as dipoles for fast click-and-release reactions. The process relies on nucleophilic aromatic substitution of aliphatic and aromatic amines with triflated sydnones. This new methodology allowed the preparation of functionalised sydnonimine probes that are otherwise difficult to prepare. These probes were then used to release a drug and a fluorescent aromatic isocyanate inside living cells.

Sydnonimines (**SIs**) belong to the mesoionic family, closely related to sydnones with the key distinction of containing a nitrogen atom at position 6. Discovered in the 1950s[1] and further developed in the 1970s for their biological properties, some of them have been approved as drugs.[2] Renewed interest in **SIs** has recently emerged due to their ability to undergo chemoselective cycloaddition reactions with strained alkynes, termed SPSIC (for Strained Promoted SydnonImine Cyclooctyne Cycloaddition).[3] This reaction mechanism involves a two-step process: a [3+2] cycloaddition followed by a retro-Diels–Alder, yielding a pyrazole clicked product and an isocyanate released product. SPSIC exhibits promise as a novel bioorthogonal click-and-release tool applicable in target fishing,[4] bioconjugation,[5] cell imaging[6] and drug release in complex biological media.[7] The speed of the reaction is remarkably affected by the substituents at position 6 of the **SI** core: the rate constant of the SPSIC reaction can be enhanced by 6 orders of magnitude simply by replacing electron-withdrawing groups by electron donating groups at this position (Scheme 1).[8] Our group has recently reported on novel **SIs** bearing alkyl or aryl groups at position 6 exhibiting remarkable kinetics for the SPSIC reaction



Scheme 1 SPSIC reaction and synthetic routes to 6-*N*-alkyl and 6-*N*-aryl-**SI**.

(k up to 1000 M^{-1} s^{-1}).[8] The synthesis of these compounds involves either reductive amination or cyclodehydration of nitrosoamides as key steps (Scheme 1). To expand the scope of synthetically accessible **SIs**, we aimed to develop a method enabling direct modification at position 6 *via* nucleophilic aromatic substitution (S_NAr) of sydnones with amines, which are accessible both synthetically and commercially. Such a method would enable **SIs** to be obtained in a single step with the possibility of significant structural diversity.

The oxygen at position 6 of sydnones is known for its poor nucleophilicity and only very strong electrophiles are able to react with it.[9] Nonetheless, aromatic nucleophilic substitutions at this position were reported in 1985 after the activation of this oxygen atom by Tf_2O and the addition of a malononitrile carbanion.[10] This reaction was recently extended to other activated methylene nucleophiles by the group of A. Schmidt to synthesise and study a series of sydnone methide compounds.[11] However, to date no implementation of this methodology has been reported for the introduction of alternative nucleophiles. Inspired by this work, we hypothesised that

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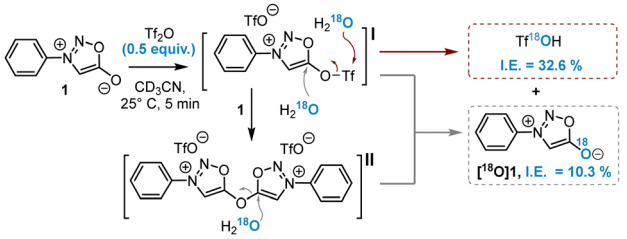


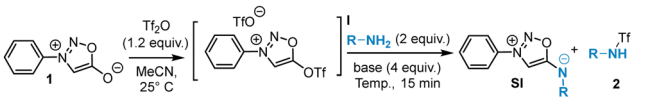
Scheme 2 Reaction of sydnones with Tf_2O. I.E.: isotopic enrichment.

amines could serve as nucleophiles in this reaction, offering a new route to **SIs** (Scheme 1). According to literature data,[10] the reaction of sydnones with Tf_2O yields a mixture of sydnone 5-triflates **I** and bis-sydnone ethers **II**, found to be very sensitive towards minute traces of water. Both species are in principle able to react with nucleophiles. Prior to investigating the reaction with amines, we initiated a study to elucidate this activation step through quenching experiments with isotopically labelled $H_2{}^{18}O$ (Scheme 2).

The activation of sydnone **1** was carried out using 0.5 equiv. of Tf_2O to favour the formation of intermediate **II**. As a consequence, after quenching with $H_2{}^{18}O$ a maximum of 50% isotopic enrichment (I.E.) of labelled sydnone **[^{18}O]1** or $Tf^{18}OH$ could be expected. ^{19}F-NMR monitoring showed complete consumption of Tf_2O before addition of $H_2{}^{18}O$, while ^{1}H-NMR indicated the formation of two new sydnone species (Fig. S1, ESI†). ^{19}F-NMR and HRMS analysis just after the addition of $H_2{}^{18}O$ showed $Tf^{18}OH$ as the major labelled product, which can be generated only from the hydrolysis of **I** (Fig. S2, ESI†). ^{18}O-labelled sydnone **[^{18}O]1**, formed by S_NAr reaction either on **I** or **II**, represented only a quarter of the total labeled compounds. Control experiments confirmed that no ^{18}O exchange occurred either on sydnone or on the triflate salt under the reaction conditions (Fig. S3, ESI†). These results proved that **I** is the main active species formed during the reaction and, as expected, that the S_NAr reaction on **I** competes with the nucleophilic attack on the sulphur atom of the triflate moiety. This side reaction could pose a significant challenge when amine nucleophiles are used on **I**.

From these findings, we explored the reaction with aliphatic and aromatic amines using benzylamine and aniline as model substrates (Table 1). The S_NAr reactions of both model amines on triflate **I** were found to be very fast: completion of the reactions was observed after 10 min even at room temperature showing the high reactivity of triflate **I**. Gentle heating at 40 °C allowed a significant increase of the yield, but higher temperatures were detrimental to the reaction due to the degradation of the substrates. The presence of a base is beneficial to the reaction, TEA being the most effective. Finally, the influence of the solvent was investigated revealing CH_3CN as the most suitable. Despite these efforts, the yields plateaued around 50% due to the side reaction generating triflated amines **2** and to purification issues. Attempts to use alternative activation methods for sydnones besides Tf_2O (Ts_2O, Ns_2O…) to minimize the formation of **2** were unsuccessful.

Table 1 Optimization of the reaction



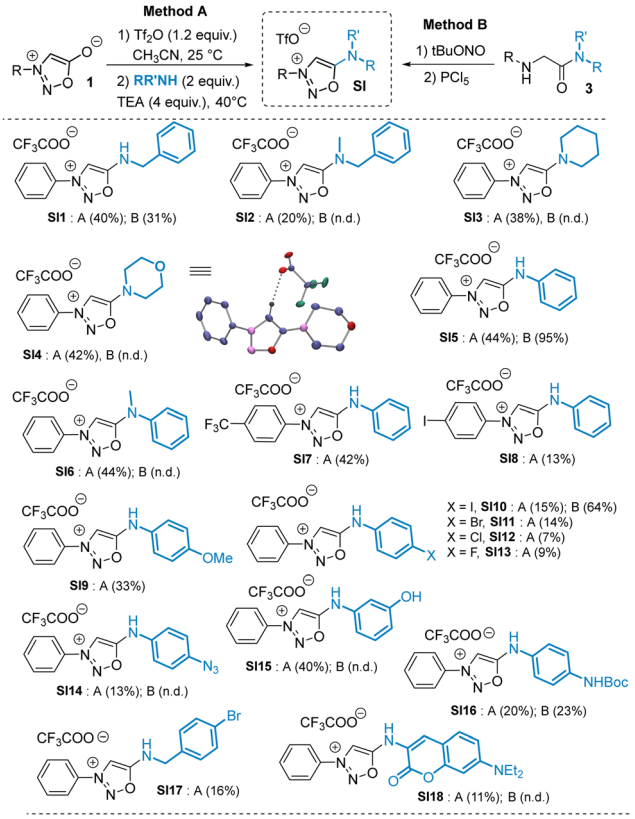
Entry	Solvent	RNH_2	Base	Temp. (°C)	**SI** (yield)[a]	**2** (yield)[b]
1	MeCN	$BnNH_2$	—	25	30%	40%
2	MeCN	$BnNH_2$	DiPEA	25	31%	24%
3	MeCN	$BnNH_2$	DiPEA	40	39%	39%
4	MeCN	$BnNH_2$	DiPEA	60	n.d.	n.d.
5	MeCN	$BnNH_2$	DABCO	40	47%	3%
6	MeCN	$BnNH_2$	Pyridine	40	n.d.	n.d.
7	**MeCN**	**$BnNH_2$**	**TEA**	**40**	**50%**	**36%**
8	DMF	$BnNH_2$	TEA	40	n.d.	n.d.
9	THF	$BnNH_2$	TEA	40C	45%	n.d.
10	$CHCl_3$	$BnNH_2$	TEA	40	42%	11%
11	Toluene	$BnNH_2$	TEA	40	50%	n.d.
12	**MeCN**	**$PhNH_2$**	**TEA**	**40**	**44%**	19%
13	MeCN	$PhNH_2$	TEA	60	41%	25%
14	MeCN	$PhNH_2$	TEA	80	40%	17%
15	THF	$PhNH_2$	NaH	40	n.d.	n.d.
16	THF	$PhNH_2$	*t*BuOK	40	n.d.	n.d.

[a] Crude yields determined by NMR for entries 1–11 and isolated yields for entries 12–16. n.d. = not detected. [b] Crude yields determined by UPLC-MS.

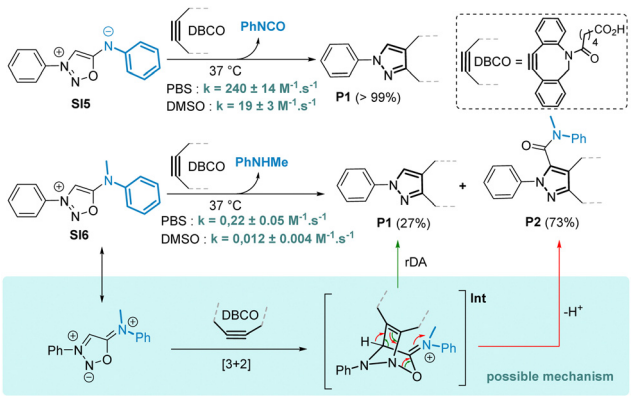
Despite the challenges, we were intrigued by the potential synthetic utility of this reaction and explored its scope further (Scheme 3).

Smooth S_NAr reactions were observed with both aliphatic and aromatic amines bearing electron rich or electron-poor substituents. To compare the S_NAr reaction (method A) with our previously reported cyclodehydration approach (method B),[8] we synthesized a series of **SIs** using both methods. The S_NAr procedure proved to be a valuable complement to method B and allowed the synthesis of **SIs** otherwise difficult or impossible to prepare. Although the S_NAr approach gave low to moderate yields, it tolerates a variety of functional groups such as phenol, azide and carbamate, which are incompatible with method B. Interestingly, secondary amines are also good substrates for the S_NAr reaction, yielding unprecedented compounds bearing an exocyclic tertiary amine at position 6 of the sydnonimine core. These derivatives, inaccessible *via* method B, are not mesoionics and thus represent novel chemical species for exploration in cycloaddition reactions with strained alkynes. We thus conducted kinetic experiments with model substrates **SI5** and **SI6**, which differ only in the presence of a methyl group, in the presence of the cyclooctyne DBCO (Scheme 4).

As anticipated, the kinetic behaviour of the two compounds exhibited drastic differences. Compound **SI6** reacted 923 times slower in reaction with DBCO in PBS compared to **SI5**. In addition, the reaction of DBCO with **SI6** produced not just one but two pyrazole products **P1** and **P2**. Both pyrazoles were formed at the same speed with a kinetic constant of $k = 0.22 \pm 0.05\ M^{-1}\ s^{-1}$ in PBS, suggesting that the mechanism involves a common intermediate whose formation is the rate determining step. We postulate that the formation of intermediate **Int** resulting from a [3+2] cycloaddition on **SI6** can undergo a

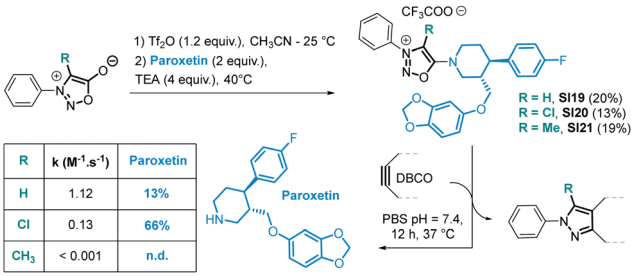


Scheme 3 Scope of the reaction. Reaction conditions: (1) TF_2O 1.2 equiv., MeCN – 25 °C, (2) RR′NH 2.0 equiv. TEA 4 equiv. MeCN – 40 °C. n.d. = not detected. The trifluoroacetate anion comes from the reversed phase purification.



Scheme 4 Reaction of sydnonimines **SI5** and **SI6** with DBCO. Proposed mechanism for the formation of **P1** and **P2** from **SI6**. The rate constants were determined by UV monitoring, [**SI5**] = [**SI6**] = 100 μM, DBCO 1.0 equiv. in 0.1 M PBS pH = 7.4 or in DMSO. Yields of pyrazoles were estimated by ^{1}H NMR monitoring in DMSO-d_6.

rDA reaction to form pyrazole **P1**. On the other hand, **Int** can also evolve after proton abstraction to generate pyrazole **P2** (Scheme 4). As the formation of pyrazole **P1** is concomitant with the release of the secondary amine attached in position 6 of the **SI** core, a possible application of this chemistry may lie in the release of drugs attached to this position. To evaluate this



R	k (M^{-1}.s^{-1})	Paroxetin
H	1.12	13%
Cl	0.13	66%
CH_3	< 0.001	n.d.

Scheme 5 Release of paroxetin from **SI19–21**; monitoring was carried out by UPLC, 4-nitro-2-fluorophenol as an internal standard. Reaction conditions: [**SI**] = 100 μM, DBCO 1.0 equiv. in PBS pH = 7.4, 25 °C.

hypothesis, we conducted proof-of-concept experiments. **SI19**, obtained in 20% yield using the S_NAr reaction, was designed to release the antidepressant drug Paroxetin upon reaction with DBCO (Scheme 5). The SPSIC reaction was conducted in aqueous conditions at pH 7.4, 25 °C and monitored by UPLC. The results confirmed a significant rate constant of k = 1.12 ± 0.21 M^{-1} s^{-1} but incomplete release of the drug over time (~13% release, Fig. S13, ESI†). This lack of release efficiency may be attributed to the formation of $\mathbf{Int_2}$ that cannot undergo the rDA-promoted release of the Paroxetin. To avoid this, we then synthesised **SI20** and **SI21** substituted by a Cl atom and a methyl group, respectively, at position 4 of the **SI** core in order to prevent the proton abstraction step. Although **SI21** proved to be unreactive, **SI20** allowed slow but efficient drug release upon reaction with DBCO (Scheme 5 and Fig. S16, ESI†). Although improvements are clearly required, these **SI**-protected drugs might be interesting in future bioorthogonal decaging strategies.

We then focused our interest on the coumarin-containing compound **SI18**, which should generate a fluorescent isocyanate upon SPSIC reaction. Interestingly, we found that **SI18** reacted as a fluorogenic probe with DBCO to form an isocyanate, the fluorescence of which is slightly higher than the starting sydnonimine (turn-on around × 5, Fig. 1).

The pK_a of compound **SI18** was determined at 4.8 ± 0.1 (Fig. S8 and S10, ESI†). Accordingly, **SI18** is mainly deprotonated at physiological pH generating a highly reactive mesoionic species. This was confirmed by fluorescence monitoring of the SPSIC reaction of **SI18** with DBCO: the kinetic constant was found to be k = 533 ± 27 M^{-1} s^{-1} meaning complete click-and-release reaction within minutes when the compounds are used in μM concentrations (Fig. S12, ESI†). We then studied whether this probe may be used to release fluorescent isocyanates inside living cells (Fig. 2A). A549 cells were incubated for 2 h with **SI18**. After washing, SPSIC reaction was initiated by the addition of DBCO (250 μM). Careful monitoring using wide field microscopy (Fig. S17 and S18, ESI†) over 2 h showed a 4-fold increase in fluorescence signal intensity achieved after 30 min reaction time (Fig. 2B). The confocal microscopy images shown in Fig. 2D indicate a significant increase in fluorescence intensity after addition of DBCO. Interestingly, this labelling was resistant to several successive washing steps (Fig. 2C and Fig. S19, ESI†), suggesting irreversible fluorescent labelling of the cells.

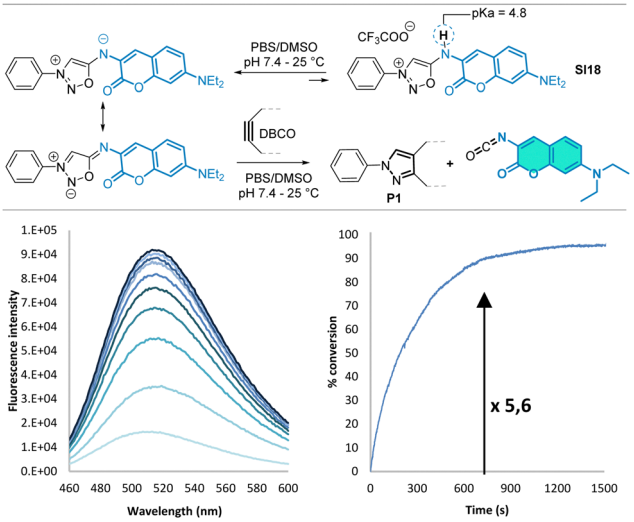


Fig. 1 SPSIC reaction of probe **SI18** with DBCO under physiological conditions. Kinetics were monitored by fluorescence, λ_{ex} = 404 nm, λ_{em} = 510 nm. Reactions conditions: 37 °C, [**SI18**] = 10 μM, DBCO 1.5 equiv. in PBS pH = 7.4.

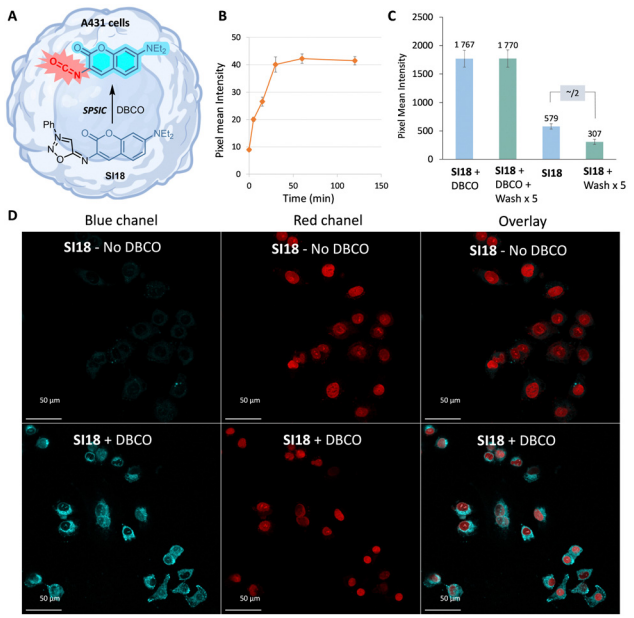


Fig. 2 SPSIC reaction inside living A549 cells. Cells were washed before the addition of DBCO. (A) Schematic representation of isocyanate release. (B) Fluorescence intensity evolution with time in A549 cells after the addition of DBCO. (C) Fluorescence intensity after 2 h in the presence or absence of DBCO and with or without cell washing. (D) Confocal microscopy images of cells treated with probe **SI18** in the presence or absence of DBCO. Conditions: [**SI18**] = 50 μM, incubation for 2 h then wash with PBS, addition of [DBCO] = 20 μM during 1 h.

In the meantime, the same washing process induced a significant drop of the fluorescence signal intensity in the case of untreated **SI18**. Altogether, these results confirmed the effective biorthogonal release of the coumarin-isocyanate derivative inside the cells leading to persistent labeling of the cells.

In summary, we have developed a new method for synthesizing sydnonimines that were previously difficult to obtain. Although the yields need improvement, this method's simplicity and tolerance to functional groups make it advantageous compared to existing methods. We have successfully synthesized unique **SIs** with tertiary amines at position 6, tested them in cycloaddition reactions with DBCO, and explored potential applications in drug release. Additionally, the S_NAr reaction allowed access to a fluorogenic probe for efficient fluorescence labeling inside living cells. Such compounds allow the release of fluorescent isocyanates in cells, which can be exploited to visualize bionucleophiles and to permanently label living cells holding promise for advancing our understanding of cellular processes and protein dynamics.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1 P. Brookes and J. Walker, *J. Chem. Soc.*, 1957, 4409–4416.
2 E. Y. Khmel'nitskaya, V. I. Levina, L. A. Trukhacheva, N. B. Grigoriev, V. N. Kalinin, I. A. Cherepanov, S. N. Lebedev and V. G. Granika, *Russ. Chem. Bull.*, 2004, **53**, 2840–2844.
3 S. Bernard, D. Audisio, M. Riomet, S. Bregant, A. Sallustrau, L. Plougastel, E. Decuypere, S. Gabillet, R. A. Kumar, J. Elyian, M. N. Trinh, O. Koniev, A. Wagner, S. Kolodych and F. Taran, *Angew. Chem., Int. Ed.*, 2017, **56**, 15612–15616.
4 S. Saidjalolov, E. Braud, Z. Edoo, L. Iannazzo, F. Rusconi, M. Riomet, A. Sallustrau, F. Taran, M. Arthur, M. Fonvielle and M. Etheve-Quelquejeu, *Chem. – Eur. J.*, 2021, **27**, 7687–7695.
5 I. Dovgan, A. Hentz, O. Koniev, A. Ehkirch, S. Hessmann, S. Ursuegui, S. Delacroix, M. Riomet, F. Taran, S. Cianférani, S. Kolodych and A. Wagner, *Chem. Sci.*, 2020, **11**, 1210–1215.
6 M. Riomet, K. Porte, A. Wijkhuisen, D. Audisio and F. Taran, *Chem. Commun.*, 2020, **56**, 7183–7186.
7 (*a*) Z. Shao, W. Liu, H. Tao, F. Liu, R. Zeng, P. A. Champagne, Y. Cao, K. N. Houk and Y. Liang, *Chem. Commun.*, 2018, **54**, 14089–14092; (*b*) M. Feng, L. Madegard, M. Riomet, M. Louis, P. A. Champagne, G. Pieters, D. Audisio and F. Taran, *Chem. Commun.*, 2022, **58**, 8500–8503.
8 M. Ribéraud, K. Porte, A. Chevalier, L. Madegard, A. Rachet, A. Delaunay-Moisan, F. Vinchon, P. Thuéry, G. Chiappetta, P. A. Champagne, G. Pieters, D. Audisio and F. Taran, *J. Am. Chem. Soc.*, 2023, **145**(4), 2219–2229.
9 S. Wiechmann, T. Freese, M. H. H. Drafz, E. G. Hübner, J. C. Namyslo, M. Niegerb and A. Schmidt, *Chem. Commun.*, 2014, **50**, 11822–11824.
10 S. Araki, J. Mizuya and Y. Butsugan, *J. Chem. Soc., Perkin Trans. 1*, 1985, 2439–2441.
11 S. Mummel, F. Lederle, E. G. Hübner, J. C. Namyslo, M. Nieger and A. Schmidt, *Angew. Chem., Int. Ed.*, 2021, **60**, 18882–18887.

